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The *MTHFR* C677T Polymorphism, Estrogen Exposure and Breast Cancer Risk: A Nested Case-control Study in Taiwan

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Abstract. *Background: We evaluated the effects of the MTHFR C677T polymorphism and its interaction with estrogen exposure on breast cancer risk in a nested case-control study conducted in Taiwan. Materials and Methods: A total of 88 histologically confirmed breast cancer cases and 344 cancer-free controls were recruited between July 1992 and December 2000. The MTHFR C677T genotype was determined by a PCR-RFLP-based assay. All subjects completed in-person interviews. Results: There was a significant trend of breast cancer in relation to prolonged exposure to estrogens prior to the first full-term pregnancy (FFTP)(p for trend=0.0015). In contrast, there was no statistically significant association between the risk of breast cancer and the MTHFR C677T genotype. However, a significantly elevated risk of breast cancer predisposed by the MTHFR 677T variant genotype (CT and TT) was observed in women with prolonged exposure to estrogens prior to FFTP (adjusted OR=4.98, 95% CI=2.00-12.43).Conclusion: The results of this study suggest that the MTHFR 677T variant genotype per se may have no overall association with breast cancer risk, but a sizable association could be observed in the presence of relevant environmental exposure.*

Epidemiological evidence has suggested that diminished folate status may increase the risk for several cancers, including breast cancer (1). Folate is involved in DNA methylation, synthesis and repair. Low intake of folate can reduce the availability of S-adenosylmethionine for DNA methylation and thereby influence gene expression (1). Folate deficiency may also result in abnormal DNA synthesis due to excessive uracil misincorporation into human DNA, leading to chromosome breaks and disruption of DNA repair (2).

Several genes controlling folate metabolism are polymorphic. 5, 10-Methylenetetrahydrofolate reductase (*MTHFR*) is one of the enzymes involved in folate metabolism. *MTHFR* plays a central role in the provision of methyl groups by converting 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the primary circulating form of folate that serves as a substrate for the remethylation of homocysteine to methionine with subsequent production of S-adenosylmethionine (SAM), the universal donor of methyl, required for DNA methylation (3). Folate that is not converted through this pathway can be used for purine synthesis or the conversion of uracil to thymine, which is used for DNA synthesis and repair (3). Therefore, *MTHFR* is critical to both DNA methylation and DNA synthesis. 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 (4). The nucleotide 677 polymorphism results in an allozyme with 65% and 30% of the wild-type homozygote activity for heterozygotes and homozygotes of the variant allele, respectively (4). Previous studies have reported that the nucleotide 677 polymorphism of *MTHFR* was associated with a decreased risk of colorectal cancer (5), acute lymphocytic leukemia (6) and lung cancer (7). In contrast, this polymorphism was observed to enhance the risk of gastric (8) and endometrial (9) cancers. Thus, the effects of the *MTHFR* C677T polymorphism on carcinogenesis are complex, either exerting the adverse effect on DNA methylation or the advantageous influence

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Table I. *Selected hormonal and body size risk factors for breast cancer*[a].

Risk factor	Cases (n=88) No. (%)	Controls (n=344) No. (%)	OR[c,f] (95% CI[c])	Trend test *p* value
Age at menarche[b] (years)				
> 17	9 (10.7)	63 (19.8)	1.00	0.0012
14-17	60 (71.4)	223 (70.1)	3.00 (1.24-7.24)	
< 14	15 (17.9)	32 (10.1)	6.35 (2.05-19.67)	
Age at FFTP[b,c] (years)				
< 21	7 (12.5)	32 (12.9)	1.00	0.03
21-25	24 (42.9)	164 (65.9)	0.60 (0.23-1.58)	
> 25	25 (44.6)	53 (21.2)	1.94 (0.69-5.42)	
Menopausal status				
Premenopausal	51 (60.7)	199 (62.0)	1.00	
Postmenopausal	33 (39.3)	122 (38.0)	1.01 (0.40-2.54)	
Body mass index[b] (kg/m^2)				
< 21.6	18 (20.4)	85 (24.7)	1.00	0.29
21.6-26.8	44 (50.0)	175 (50.9)	1.19 (0.64-2.22)	
> 26.8	26 (29.6)	84 (24.4)	1.45 (0.72-2.91)	
Use of OC[c]				
Never	61 (73.5)	225 (71.1)	1.00	
Ever	22 (26.5)	96 (29.9)	0.83 (0.46-1.51)	
Years of early estrogen exposure[d]				
< 5	6 (10.9)	48 (20.9)	1.00	0.0015
5-10	25 (45.5)	140 (60.9)	1.34 (0.51-3.55)	
> 10	24 (43.6)	42 (18.2)	5.02 (1.68-15.02)	
Years of cumulative estrogen exposure[e]				
< 24	17 (21.0)	72 (23.1)	1.00	0.05
24-34	43 (53.1)	183 (58.6)	1.97 (0.47-8.30)	
> 34	21 (25.9)	57 (18.3)	3.65 (0.74-17.94)	

[a] Total number of cases and controls does not correspond because of missing values.
[b] Risk factors were classified according to distributions of less than the first tertile, between first and third tertile and greater than the third tertile among control subjects.
[c] FFTP, first full-term pregnancy; OC, oral contraceptives; OR, odds ratio; CI, confidence interval.
[d] Years of early estrogen exposure were estimated by the interval between age at menarche and age at first full-term pregnancy.
[e] For premenopausal women, years of cumulative estrogen exposure were defined by the interval between age at menarche and age at enrollment; for postmenopausal women, years of cumulative estrogen exposure were indicated by the interval between age at menarche and age at menopause.
[f] Odds ratios are adjusted for age at enrollment and ethnicity.

on nucleotide synthesis in determining cancer risk. It should be noted that germ-line polymorphism in *MTHFR* has not been as well-studied in relation to breast cancer risk.

Both epidemiological and animal studies have indicated that estrogens are central in breast carcinogenesis (10). Estrogens induce proliferation, but may also initiate carcinogenesis *via* metabolic activation to potentially carcinogenic catechol estrogen metabolites. The principal pathway for inactivation of catechol estrogen is *O*-methylation by catechol-*O*-methyltransferase (*COMT*) (11). While SAM is the necessary methyl donor for *COMT*-catalyzed reactions, the folate metabolic pathway largely determines the SAM level. Thus, it is possible that polymorphisms in the folate-metabolizing gene, *MTHFR*, might be interactive with estrogen exposure in determining breast cancer risk. We tested this hypothesis by genotyping our specimens from a population-based nested case-control study of breast cancer for the nucleotide 677 polymorphism of *MTHFR*.

Materials 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 and methods of screening and follow-up have been described previously (7,12). From July 1990 to June 1992, a community-based cancer screening project was carried out in seven townships. Makung, Hushi and Paihsa Townships are located on Penghu Islets. The other four townships Sanchi, Chutung, Potzu and Kaoshu, are located on the main Taiwan Island. There were 47,079 eligible males and 42,263 eligible females who were invited by letter to participate. A total of 12,026 male and 11,917 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 (12).

Study subjects. 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 85% complete (13). In the present study, when a case of female breast cancer was identified *via* data linkage, permission was sought from the hospital where the diagnosis had been made to obtain the subject's medical charts and pathology reports. Between July 1992 and December 2000, a total of 88 pathologically confirmed primary breast carcinoma patients were identified among female cohort members. Four female control subjects were matched to each case by age (±2 years), residence and date of blood sample collection (±3 months). Control subjects were free of cancer when their matched cases were diagnosed. Among control individuals, 8 subjects with insufficient blood sample were excluded from the analysis. As a consequence, there were 81 case-controls sets with 1 case matched to 4 controls, 6 sets with 1 case and 3 controls, and 1 set with 1 case and 2 controls. Therefore, the final study subjects included 88 cases and 344 controls.

Data collection. At baseline recruitment, well-trained research assistants administered a structured questionnaire to participants. The information collected from female subjects included sociodemographic characteristics, history of cigarette smoking and alcohol consumption, personal and family history of cancer, age at

Table II. *MTHFR C677T genotype distribution and allele frequency between case and control subjects and in relation to breast cancer risk.*

MTHFR[a,b] Genotype/allele	Cases No. (%)	Controls No. (%)	Crude OR[a]	Adjusted OR[c] (95% CI[a])
CC	43 (48.9)	173 (50.6)	1.00	1.00
CT	38 (43.2)	145 (42.4)	1.04	1.05 (0.65-1.70)
TT	7 (7.9)	24 (7.0)	1.18	1.21 (0.48-3.03)
CT/TT	45 (51.1)	169 (49.4)	1.06	1.07 (0.68-1.70)
Allele frequency				
C	0.70	0.72		
T	0.30	0.28		

[a] *MTHFR*, 5,10-methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.
[b] Data on *MTHFR* genotype were not available for 2 controls.
[c] Odds ratios are adjusted for age at enrollment and ethnicity.

Table III. *Joint association of years of early estrogen exposure and MTHFR C677T genotype with breast cancer risk.*

	Years of early estrogen exposure[b]			
	<=10 years		> 10 years	
MTHFR[a] genotype	Case/ Control	OR[a,c] (95% CI[a])	Case/ Control	OR (95% CI)
CC	16/92	1.00	11/27	2.34 (0.97-5.68)
CT/TT	15/95	0.91 (0.42-1.94)	13/15	4.98 (2.00-12.43)

[a] *MTHFR*, 5,10-methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.
[b] Years of early estrogen exposure were estimated by the interval between age at menarche and age at first full-term pregnancy.
[c] Odds ratios are adjusted for age at enrollment and ethnicity.

menarche and/or menopause, parity and age at first full-term pregnancy (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 subjects gave informed consent for both the interview and blood collection. This community-based cancer screening program was supported and approved by the Department of Health, Executive Yuan, Republic of China.

MTHFR genotyping. Genomic DNAs from the cases and controls were analyzed for the presence of the C to T transversion at nucleotide 677 of *MTHFR* by a PCR-based restriction fragment length polymorphism (RFLP) assay, as described by Frosst *et al.* (4). Based on the *Hinf*I RFLP analysis for *MTHFR* C677T polymorphism, a single undigested band at 198 bp represents a homozygous wild-type allele, two bands at 198- and 175-bps represent the heterozygous genotype and a single band at 175 bp represents a homozygous mutant allele. Direct sequencing was conducted for 8 PCR products to confirm that the amplified DNA was the targeted sequence of *MTHFR*.

Statistical methods. The associations between risk determinants, including hormonal risk factors and *MTHFR* genotype, and the subsequent development of breast cancer were examined using conditional logistic regression to calculate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs). Individuals homozygous for the wild-type allele were designated as the referent category for the analyses of the *MTHFR* genotype. Of particular interest was the potential interactive effect between the *MTHFR* genotype and estrogen exposure on breast cancer risk. We adopted two indices to estimate the estrogen exposure level: (a) the number of years between menarche and FFTP, and (b) cumulative years of estrogen exposure, which was calculated according to the age at menarche and age at enrollment for premenopausal women and age at menarche and age at menopause for postmenopausal women. Statistical assessment of interaction between *MTHFR* C677T polymorphism and estrogen exposure in relation to breast cancer risk was made on the basis of an additive scale by estimating the synergy index (S) (14, 15). In addition, the χ^2 test was conducted to examine the Hardy-Weinberg equilibrium. We performed all analyses using Statistical Analysis System Software (v.8.0; SAS Institute Inc., NC, U.S.A.).

Results

The mean age of the cases and the controls at the time of enrollment was 50.9 and 50.8 years, respectively. There were no major case-control differences with respect to ethnicity and residential areas. Table I presents ORs and 95% CIs for hormonal risk factors in association with breast cancer risk. Age at menarche was inversely related to risk of breast cancer (p value for linear trend test=0.0012). Women with menarchal age 14 or less had an OR of 6.35 (95% CI=2.05-19.67) relative to those who began menstruation after age 17. Delayed age at FFTP was associated with a significantly elevated risk of breast cancer (p for trend=0.03). Compared to parous women whose FFTP occurred before the age of 21, those who had their FFTP after age 24 demonstrated an OR of 1.94 (95% CI=0.69-5.42). There were no significant case-control differences with respect to menopausal status, body mass index and use of oral contraceptives. More interestingly, there was a significant trend of breast cancer risk in relation to cumulative years of estrogen exposure (p for trend=0.05). Women who had been exposed to estrogen for more than 34 years had an OR of 3.65 (95% CI=0.74-17.94) relative to those who had been exposed to estrogen for less than 24 years. Moreover, prolonged duration from

menarche to FFTP was substantially associated with enhanced risk of breast cancer (*p* for trend=0.0015). The OR for breast cancer in women with a longer duration (> 10 years) from menarche to FFTP relative to those with a shorter duration (< 5 years) was 5.02 (95% CI=1.68-15.02).

The frequencies of *MTHFR* 677T allele and C677T genotype by case-control status and the association between the *MTHFR* genotype and breast cancer risk are presented in Table II. The *MTHFR* 677T allele frequencies were 0.30 and 0.28, respectively, in cases and controls. The distribution of genotype among controls was in agreement with that predicted under the condition of the Hardy-Weinberg equilibrium (χ^2 test, p=0.38). We observed that 7 (7.9%) cases and 24 (7.0%) controls were homozygous for the C677T variant allele, resulting in an adjusted OR of 1.21 (95% CI=0.48-3.03). The *MTHFR* 677T variant genotype, including the heterozygous and homozygous categories, was not statistically significantly associated with breast cancer risk (adjusted OR=1.07, 95% CI=0.68-1.70).

We then evaluated the joint associations of the *MTHFR* C677T genotypes and estrogen exposure with breast cancer risk to test our hypothesis. Compared with women who had a shorter duration (≤10 years) from menarche to FFTP and had the *MTHFR* 677CC genotype, those who had a longer duration (> 10 years) and had the *MTHFR* 677CC genotype showed an adjusted OR of 2.34 (95% CI=0.97-5.68). Furthermore, a significantly increased risk of breast cancer was observed among subjects who had a longer duration (>10 years) of estrogen exposure prior to FFTP and had the *MTHFR* 677T variant genotype (adjusted OR=4.98, 95% CI=2.00-12.43) (Table III). There appeared to be an additive interaction between the C677T polymorphism in the *MTHFR* gene and duration of estrogen exposure in relation to breast cancer risk (S index=3.18, 95% CI=0.52-19.51).

Discussion

Chinese women historically have a lower risk of breast cancer compared to their counterparts in Western countries. This population-based nested case-control study demonstrated a high degree of reproducibility of most established menstrual and reproductive breast cancer risk factors of Western populations in a low-risk country. Henderson and Feigelson (10) have interpreted these risk factors under a unifying hypothesis that endogenous estrogens are central to breast carcinogenesis. In our study, cumulative exposure to estrogens was associated with an increased risk of breast cancer. Of particular note, the risk of breast cancer was strongly determined by a prolonged period between menarche and FFTP (Table I). The interval between menarche and FFTP could be prolonged as a result of earlier menarche and/or delayed FFTP. It is known that women with an earlier menarche tend to have higher estrogen levels than those with a later menarche (16). Furthermore, pregnancy at a younger age is associated with a favorable estrogen profile, which drastically reduces the presence of undifferentiated/vulnerable breast cells, differentiates terminal end buds to lobules, and/or reduces the pool of estrogen receptor-positive cells (17). Indeed, Colditz and Frazier (18) have argued that the time between menarche and FFTP had the highest rate of breast tissue aging, and this time period was the time when the breast tissue was most vulnerable to mutagenesis. Overall, our results, together with other study findings, indicate that, although Asian women show an average 20% reduction in estradiol compared with Western women (19), breast cancer in this low-incidence area remains highly hormone-dependent, as in high-risk areas.

Epidemiological evidence has implicated diminished folate status in the development of several cancers, including breast caner (1). These observations may be explained by the crucial role of folate as the donor of one-carbon groups in both DNA methylation and nucleotide synthesis. *MTHFR* is a key enzyme in folate metabolism and irreversibly catalyzes 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the donor for the remethylation of homocysteine to methionine, the precursor for the universal methyl donor. If not reduced to 5-methylenetetrahydrofolate by *MTHFR*, 5, 10-methylenetetrahydrofolate can transfer its methylene group to dUMP to synthesize dTMP or may contribute to purine synthesis (3). A defect in *MTHFR* could thus influence both DNA methylation and DNA synthesis, so its association with cancer risk is of great interest. A variant of the human *MTHFR* gene, that results in an alanine to valine substitution, has been described at bp 677. This mutation codes for a thermolabile enzyme with reduced *MTHFR* activity (4). Previous reports indicated that the *MTHFR* C677T variant genotype was associated with a decreased risk of colorectal cancer (5), acute lymphocytic leukemia (6) and lung cancer (7). In contrast, other studies have implicated the C677T variant genotype of *MTHFR* in increased risk of gastric (8) and endometrial (9) cancers. The *MTHFR* C677T polymorphism thus may have a dual effect in determining cancer risk, either the adverse effect on DNA methylation or the advantageous influence on nucleotide synthesis.

MTHFR polymorphisms have not been adequately investigated in relation to breast cancer risk. We found, in this nested case-control study, that the variant *MTHFR* genotypes 677TT and 677CT were not statistically significantly associated with risk of breast cancer when compared with the 677CC genotype. Our results are in accordance with the findings from a case-control study

carried out in China (20); but are in contrast to the results from a case-control study conducted in the United Kingdom (21). Discrepant results among studies investigating *MTHFR* polymorphisms and the risk of breast cancer may be due to differences in the ethnicity of the study subjects and other breast cancer risk parameters to be controlled for. In addition, whether or not taken into account, dietary folate intake in the *MTHFR* analysis between the studies could also have affected the results, given the study findings that the cancer risk associated with the *MTHFR* 677T variant genotype varies according to folate status (7,22).

More interestingly, we observed an increased risk of breast cancer for the *MTHFR* 677T variant genotype among women with prolonged exposure to estrogen before FFTP (Table III), a critical period when breast tissue is vulnerable to mutagenesis (18). It has been noted that estrogens may initiate breast carcinogenesis *via* metabolic activation to potentially carcinogenic catechol estrogen metabolites (13). The principal pathway for inactivation of catechol estrogen is O-methylation by *COMT*, which involves SAM as the necessary methyl donor for *COMT* catalyzed reactions (11). While methionine is the direct precursor to SAM, *MTHFR* catalyzes the biologically irreversible reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor for methionine synthesis from homocysteine (3). Thus, polymorphism in the *MTHFR* gene that affects methionine synthesis and consequent *COMT* catalyzed reactions, in combination with prolonged exposure to estrogens during a critical period, could be associated with increased breast cancer risk.

In conclusion, the present study provides additional evidence that the major hormonal risk factors of Western societies also define a high-risk profile in Chinese women. With respect to individual susceptibility, our findings suggest that the *MTHFR* 677T variant genotype *per se* may have no overall association with breast cancer risk, but a sizable association could be observed in the presence of relevant environmental exposure, such as prolonged exposure to estrogens within the critical period of breast tissue vulnerability. Although our results are consistent with biologically plausible interactions, the number of subjects in this study was relatively small. Further studies are needed to elucidate the joint effect of polymorphisms in genes controlling for folate metabolism and estrogen exposure in breast cancer etiology.

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References

1 Kim YI: Folate and carcinogenesis: Evidence, mechanisms, and implications. J Nutr Biochem *10*: 66-88, 1999.
2 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.
3 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.
4 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, Denheijer M, Kluijtmans LAJ, Vandenheuvel LP and Rozen R: A candidate genetic risk factor for vascular-disease - a common mutation in methylenetetrahydrofolate reductase. Nature Genet *10*: 111-113, 1995.
5 Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC and Hunter DJ: A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res *56*: 4862-4864, 1996.
6 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.
7 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.
8 Shen HB, Xu YC, Zheng YX, Qian Y, Yu RB, Qin Y, Wang XR, Spitz MR and Wei QY: Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: A case-control study. Int J Cancer *95*: 332-336, 2001.
9 Esteller M, Garcia A, Martinez-Palones JM, Xercavins J and Reventos J: Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (*MTHFR*) genes and endometrial cancer susceptibility. Carcinogenesis *18*: 2307-2311, 1997.
10 Henderson BE and Feigelson HS: Hormonal carcinogenesis. Carcinogenesis *21*: 427-433, 1996.
11 Yager JD and Liehr JG: Molecular mechanisms of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol *36*: 203-232, 1996.
12 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.
13 Yu MW, Yang YC, Yang SY, Cheng SW, Liaw YF, Lin SM and Chen CJ: Hormonal markers and hepatitis B virus-related hepatocellular carcinoma risk: a nested case-control study among men. J Natl Cancer Inst *93*: 1644-1651, 2001.
14 Rothman KJ: Modern Epidemiology. Boston, MA: Little, Brown and Company, pp.322-326, 1986.
15 Hosmer DW and Lemeshow S: Confidence interval estimation of interaction. Epidemiology *3*: 452-456, 1992.
16 Apter D, Reinila M and Vihko R: Some endocrine characteristics of early menarche, a risk factor for breast-cancer, are preserved into adulthood. Int J Cancer *44*: 783-787, 1989.

17 Russo J, Tay LK and Russo IH: Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res Treat *2*: 5-73, 1982.

18 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.

19 Bernstein L, Yuan JM, Ross RK, Pike MC, Hanisch R, Lobo R, Stanczyk F, Gao YT and Henderson BE: Serum hormone levels in pre-menopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control *1*: 51-58, 1990.

20 Shrubsole MJ, Gao YT, Cai QY, 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.

21 Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, Baird JOC, 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.

22 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.

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