Breast Cancer Res Treat (2010) 123:885–893
DOI 10.1007/s10549-010-0804-4

EPIDEMIOLOGY

Genetic polymorphisms in folate and alcohol metabolism and breast cancer risk: a case–control study in Thai women

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Received: 23 December 2009 / Accepted: 10 February 2010 / Published online: 24 February 2010

Abstract Dietary folate as well as polymorphic variants in one-carbon metabolism genes may modulate risk of breast cancer through aberrant DNA methylation and altered nucleotide synthesis and repair. Alcohol is well recognized as a risk factor for breast cancer, and interactions with one-carbon metabolism has also been suggested. The purpose of this study is to test the hypothesis that genetic polymorphisms in the folate and alcohol metabolic pathway are associated with breast cancer risk. Twenty-seven single nucleotide polymorphisms (SNPs) in the *MTR*, *MTRR*, *MTHFR*, *TYMS*, *ADH1C*, *ALDH2*, *GSTP1*, *NAT1*, *NAT2*, *CYP2E1 DRD2*, *DRD3*, and *SLC6A4* were genotyped. Five hundred and seventy patients with histopathogically confirmed breast cancer and 497 controls were included in the present study. Association of genotypes with breast cancer risk was evaluated using multivariate logistic regression to estimate odds ratios (OR) and their 95% confidence intervals (95% CI). Increased risk was observed for homozygotes at the *MTR* SNPs (rs1770449 and rs1050993) with the OR = 2.21 (95% CI 1.18–4.16) and OR = 2.24 (95% CI 1.19–4.22), respectively. A stratified analysis by menopausal status indicated the association between the *NAT2* SNP (rs1799930) and breast cancer was mainly evident in premenopausal women (OR 2.70, 95% CI 1.20–6.07), while the *MTRR* SNP (rs162049) was significant in postmenopausal women (OR 1.61, 95% CI 1.07–2.44). Furthermore, SNPs of the genes that contribute to alcohol behavior, *DRD3* (rs167770), *DRD2* (rs10891556), and *SLC6A4* (rs140701), were also associated with an increased risk of breast cancer. No gene–gene or gene–environment interactions were observed in this study. Our results suggest that genetic polymorphisms in folate and alcohol metabolic pathway influence the risk of breast cancer in Thai population.

Keywords Breast cancer · Folate · Alcohol · Metabolizing enzyme · Single nucleotide polymorphisms

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Introduction

Breast cancer is the second most common cancer in Thai women and the incidence is still increasing [1]. A wide variety of genetic damage induced by endogenous metabolites and exogenous hazards may contribute to the etiology of breast cancer. Folate is an important nutrient required for DNA synthesis, and it is also involved in the methionine metabolic pathway, which is crucial for DNA methylation [2]. At least 30 different enzymes are involved in this complex pathway including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and thymidylate synthase (TYMS). Defects or polymorphic variations in the folate metabolic pathway may influence cancer susceptibility [3].

Evidence level seems to be high that alcohol increases risk of breast cancer [4]. A pooled analysis of 4,335 breast cancer cases and >300,000 controls suggested that intake of 2–5 drinks/day increased risk by roughly 40% [5]. The underlying mechanisms are not firmly established [6] but may include an influence on circulating levels of estrogens [7], immune function, enhanced permeability of chemical

carcinogens, decreased absorption of essential nutrients [8], or through metabolism of alcohol to acetaldehyde, a known carcinogen [9].

A relative folate deficiency may develop in individuals who chronically consume more than moderate amounts of alcohol because of the negative effects of alcohol on folate metabolism, including malabsorption, increased excretion, or enzymatic suppression [10]. The potential for high folate intake to counteract the elevated risk of breast cancer associated with alcohol consumption has been illustrated by data from a number of studies [11–14].

To further investigate the role of these pathways in mammary carcinogenesis, we analyzed the association between breast cancer and 27 SNPs in 13 key genes involved in one-carbon and alcohol metabolism on 570 cases and 497 controls in Thai women. We also investigated gene–gene and gene–environment interactions.

Materials and methods

Study population

Cases were all new incident breast cancer patients histologically diagnosed at the National Cancer Institute in Bangkok and at the hospital in Khon Kaen province of North Eastern of Thailand during the period of May 2002–March 2004 and August 2005–August 2006, with a participation rate of 99.6% (600/602). Controls were randomly selected from healthy women who visited patients admitted to the same hospitals for diseases other than breast or ovarian cancer. All of the 642 control individuals were recruited during the same study period as the case ascertainment. The participation rate among visitors who were asked to participate was 98.9% (642/649). Informed consent was obtained from all participants and a structured questionnaire was administered by trained interviewers to collect information on demographic and anthropometric data, reproductive and medical history, residential history, physical activity and occupation as well as diet (see Table 1). Lifestyle exposure parameters were reported as follows: tobacco smoking: less than or equal to 6 months of smoking in life, or if she smokes longer than 6 months, the sum of cigarettes smoked is less than or equal to 50 per 6 months (non-smoker) versus more than 50 cigarettes over a 6-month period (smoker); involuntary tobacco smoking: less versus more than or equal to 1 h of exposure per day; alcohol consumption: less versus more than or equal to once a week for at least 6 months. Approximately 7 ml of blood were collected from participants, but 30 cases and 145 controls refused to give blood samples. In total, blood samples of 570 cases and 497 controls were included in the genotype analysis, resulting in a participation rate of 95.0% (cases) and 77.4% (controls). The study was approved by the ethical review committee for research in human subjects, Ministry of Public Health, Thailand and by the ethics committee of National Cancer Center, Japan.

Genotyping analysis

Genomic DNA was isolated from buffy coats using a QIAmp DNA blood kit (Qiagen, Hilden, Germany). DNA concentrations were measured by PicoGreen dsDNA qualification kits (Molecular Probes, Leiden, The Netherlands). All SNPs were analyzed by TaqMan 5′ nuclease assay using the ABI PRISM 7900 HT Sequence Detection System (Applide Biosystems LLC, Foster city, CA, USA). Oligonucleotide primers and the dual labeled allele specific probes were designed by ABI. PCR were performed in 384-well plates with each plate containing four control samples. A set of three 384-well plates were prepared to accommodate 570 cases and 497 control subjects and used for genotyping. Genomic DNA (5 ng) was amplified in a total volume of 5 μl in the presence of 100 μM of each of the dNTPs, 3 pmols of each of appropriate primers, 2 pmols of each of the corresponding dual labeled probes, and 0.025 units of Taq DNA polymerase. PCR cycling consisted of 40 cycles at 94°C for 15 s, 55–60°C for 15 s, and 72°C for 15 s. The results of 5% blindly repeated samples were at least 99% concordant each other. Genotyping success rate for individual polymorphisms averaged 95%.

Statistical analyses

Hardy–Weinberg equilibrium (HWE) testing was used as one of the measures for a quality control for genotyping, and allele and genotype frequencies were calculated. The multivariate logistic regression analyses were applied to evaluate differences in genotype distributions, and the odds ratios (OR) and their 95% confidence intervals (CI) were calculated after adjustment for the following covariates: age, body mass index (BMI), smoking, pregnancy and breast feeding, family history of breast cancer in the first-degree relatives, education, and menopausal status. Alcohol consumption was not included, because it was not associated with the breast cancer in our study (Table 2). For some SNPs, additional tests were also performed by Fisher's exact test on allelic contingency table and by Cochran–Armitage trend test to confirm the observed genotype-specific associations.

A stratified analysis was performed as an exploratory, adjunct analysis. Selected strata were menopausal, pregnancy, breast feeding, oral contraceptive use, estrogen receptor, and progesterone receptor status. The adjusted ORs and 95% CIs for OR were calculated by the

Table 1 Selected characteristics of study population

Characteristics	Cases	Controls	*P* value
Age, *n* (mean ± SD)	570 (46.0 ± 10.6)	497 (43.2 ± 12.4)	<0.0001
Age at menarche, *n* (mean ± SD)	561 (14.7 ± 1.9)	496 (14.86 ± 1.8)	0.61
Age at menopause, *n* (mean ± SD)	99 (46.6 ± 5.5)	85 (47.2 ± 5.7)	0.46
Age at first pregnancy, *n* (mean ± SD)	425 (23.1 ± 5.4)	331 (22.8 ± 4.9)	0.48
Pregnancy ($n = 1{,}067$), *n* (%)			
No	145 (25.4)	166 (33.4)	0.004
Yes	425 (74.6)	331 (66.6)	
Breast feeding ($n = 736$), *n* (%)			
No	59 (14.4)	27 (8.3)	0.011
Yes	351 (85.6)	299 (91.7)	
Oral contraceptive use ($n = 1{,}061$), *n* (%)			
No	310 (54.5)	288 (58.5)	0.19
Yes	259 (45.5)	204 (41.5)	
Menopausal status ($n = 1{,}062$), *n* (%)			
Premenopausal	325 (57.4)	316 (63.7)	0.038
Postmenopausal	241 (42.6)	180 (36.3)	
Body mass index ($n = 1{,}067$), *n* (mean ± SD kg/m^2)	570 (24.1 ± 4.4)	497 (23.0 ± 4.1)	3.7×10^{-5}[a]
BMI < 20 kg/m^2 (%)	87 (15.3)	111	
20 ≤ BMI < 25	273 (47.9)	269	
25 ≤ BMI	210 (36.8)	117	
Tobacco smoking ($n = 1{,}066$), *n* (%)			
No	552 (97.0)	489 (98.4)	0.16
Yes	17 (3.0)	8 (1.6)	
Involuntary smoking ($n = 1{,}040$), *n* (%)			
No	489 (88.7)	456 (93.3)	0.013
Yes	62 (11.3)	33 (6.7)	
Alcohol consumption ($n = 1{,}067$), *n* (%)			
No	525 (92.1)	469 (94.4)	0.18
Yes	45 (7.9)	28 (5.6)	
Family history of breast cancer in first-degree relatives ($n = 1{,}067$), *n* (%)			
No	544 (95.4)	487 (98.0)	0.026
Yes	26 (4.6)	10 (2.0)	
Education ($n = 1{,}067$), *n* (%)			
≤9 years	398 (69.8)	262 (52.7)	1.1×10^{-8}
>9 years	172 (30.2)	235 (47.3)	

[a] The *P* value for the BMI was calculated by Fisher's exact test on the 2 × 3 contingency table

multivariate logistic regression analyses. In addition, gene–gene interactions and gene–environment interactions were evaluated by logistic model including an interaction term between genes and those between gene and environmental factors.

The statistical significance was defined as $P \leq 0.05$, and adjustment for multiple testing, which is absolutely necessary in the validation phase of association studies, was not performed due to an exploratory hypothesis-generating nature of the study.

All statistical analyses were carried out using the Statistical Analysis System (SAS) software Version 9.1 (SAS Institute Inc, Cary, NC), and partially the R suite (http://www.r-project.org/).

Results

Characteristics of the study population were compared by case–control status as shown in Table 1. The mean age of controls (43.2 ± 12.4 years) was significantly lower ($P < 0.01$) than that of breast cancer patients (46.0 ± 10.6 years). Pregnancy, menopausal status, breast feeding, BMI, involuntary tobacco smoking, family history of breast cancer, and

Table 2 SNPs analyzed in this study

Gene name[a]	rs number	Nucleotide exchange[b]	Annotation	MAF[c]	HWE[d]
MTR	3795708	T > C		39.44	0.213
	1770449	A > G		21.40	0.283
	1050993	G > A		21.27	0.344
MTRR	1801394	A > G	Ile22Met	31.13	0.916
	326121	C > T		38.34	0.774
	162049	G > A		48.67	0.652
	10380	C > T	His595Tyr	14.48	0.854
	327592	T > C		15.78	0.609
MTHFR	1801133	C > T	Ala222Val	13.55	0.437
	1801131	A > C	Glu429Ala	25.87	0.025
TYMS	16948322	C > T		31.70	0.212
	2298581	G > C		37.03	0.052
ADH1C	4147542	G > A		28.50	0.262
ALDH2	4646778	C > A		23.20	0.702
	671	G > A	Glu487Lys	9.05	1.000
DRD2	7117915	G > A		36.47	0.922
	10891556	G > T		16.84	0.873
DRD3	2087017	A > G		44.50	0.068
	167770	A > G		20.42	0.400
SLC6A4	140701	A > G		23.88	0.456
GSTP1	612020	C > T		8.57	0.153
	1695	A > G		26.38	0.560
NAT1	7845127	C > T		28.27	1.000
NAT2	1041983	C > T		47.81	0.648
	1799930	G > A	Arg197Gln	31.68	0.020
CYP2E1	2249695	C > T		41.12	0.776
	2031920	C > T		15.96	0.057

[a] According to the NCBI database SNP

[b] More frequent to less frequent allele

[c] Minor allele frequency

[d] *P* value for Hardy–Weinberg equilibrium is calculated in the control population by Fisher's exact test

education were different between cases and controls. However, as to oral contraceptive use, smoking, and alcohol consumption, no significant differences were found between cases and controls.

Frequencies of variant alleles among the control population are shown in Table 2. All SNP frequencies were in Hardy–Weinberg equilibrium (HWE) among controls, except two SNPs in *MTHFR* (rs1801131) and *NAT2* (rs1799930). The result of association analysis of individual SNPs is shown in Table 3. Homozygotes of minor alleles of *MTR* SNPs (rs1770449 and rs1050993) were associated with an increased risk of breast cancer with OR = 2.21 (95% CI 1.18–4.16) and 2.24 (95% CI 1.19–4.22), respectively. The SNP in *DRD3* (rs167770) was found associated with an increased risk among heterozygote carriers (OR 1.36, 95% CI 1.03–1.80). Although the *DRD3* (rs167770) SNP did not show a statistically significant association for the minor allele homozygotes, this SNP was significant when tested for allelic (OR 1.24, 95% CI 1.01–1.53; $P = 0.045$) and recessive (OR 1.38, 95% CI 1.07–1.77; $P = 0.014$) models.

A stratified analysis by menopausal status suggested tendencies that *NAT2* (rs1799930), *DRD2* (rs10891556), and *SLC6A4* (rs140701) polymorphisms are related to breast cancer risk in premenopausal women (OR 2.70, 95% CI 1.20–6.07; OR 1.62, 95% CI 1.03–2.56; and OR 1.56, 95% CI 1.01–2.41, respectively) (Table 4). Among postmenopausal women, an increased risk of breast cancer was suggested for *MTRR* (rs162049) and *DRD3* (rs167770) polymorphisms (OR 1.61, 95% CI 1.07–2.44 and OR 1.59, 95% CI 1.11–2.28, respectively).

Analyses on gene–environment interactions were performed between polymorphisms and alcohol consumption, oral contraceptive use and body mass index. They were exploratory analyses, but no strong interactions were identified (Table 5). Two-gene interactions were also analyzed between SNPs of the *DRD3* (rs167770) and other genes showing significant association individually in Table 3. None of gene–gene interactions was statistically significant (Table 6).

Further, *DRD3*, and *MTR* genotype were correlated with ER and PR status. Fifty-three percent of breast cancer cases were ER-positive tumors (167/314) and 39% were PR-positive tumors (118/301). The associations between *DRD3* (rs167770) and *MTR* (rs1770449 and rs1050993) polymorphisms and breast cancer risk were not different among the ER/PR status (data not shown).

Discussion

Genetic variation in enzymes and other proteins involved in folate and alcohol metabolisms are rational candidates for studying the impact of both genetic and environmental effects and their interactions on breast cancer risk. As a systematic candidate gene approach, we analyzed 12 SNPs in four folate metabolism genes and 15 SNPs in nine alcohol metabolism and behavior genes; these SNPs were selected by our previous study to catalog candidate genes, which are potentially subjected to gene–environment interactions with regard to cancer susceptibilities among Japanese population [15]. Only the SNPs that have minor allele frequencies higher than 5% were evaluated in the study. Of the 27 SNPs, seven suggested an association with breast cancer risk (*MTR* rs1770449 and rs1050993, *MTRR* rs162049, *DRD2* rs10891556, *DRD3* rs167770, *SLC6A4* rs140701, and *NAT2* rs1799930) in an overall or stratified analysis.

Table 3 Association between folate and alcohol metabolism genes and breast cancer

Gene name	rs number	Homozygotes of major allele		Heterozygotes				Homozygotes of minor allele			
		Ca	Co	Ca	Co	OR[a] (95% CI)	*P*-value	Ca	Co	OR[a] (95% CI)	*P*-value
MTR	3795708	231	182	252	215	0.94 (0.71–1.25)	0.677	81	81	0.70 (0.48–1.04)	0.075
	1770449	345	296	185	172	0.93 (0.71–1.23)	0.611	36	18	2.21 (1.18–4.16)	0.014
	1050993	344	299	184	172	0.94 (0.71–1.23)	0.640	36	18	2.24 (1.19–4.22)	0.012
MTRR	1801394	295	229	218	210	0.85 (0.65–1.11)	0.228	46	46	0.85 (0.53–1.37)	0.512
	326121	213	184	262	235	0.90 (0.68–1.19)	0.463	91	70	1.01 (0.68–1.50)	0.963
	162049	142	131	285	239	1.19 (0.87–1.63)	0.284	135	118	1.07 (0.75–1.55)	0.704
	10380	409	355	144	123	1.14 (0.84–1.53)	0.398	11	9	1.20 (0.48–3.00)	0.697
	327592	401	346	151	135	1.08 (0.80–1.44)	0.622	11	10	1.05 (0.43–2.58)	0.908
MTHFR	1801133	410	366	144	110	1.23 (0.91–1.68)	0.176	9	11	0.74 (0.29–1.87)	0.520
	1801131	302	258	223	206	0.94 (0.72–1.23)	0.666	38	23	1.57 (0.89–2.79)	0.121
TYMS	16948322	246	234	249	200	1.14 (0.87–1.50)	0.336	65	55	1.27 (0.83–1.94)	0.282
	2298581	242	201	244	205	0.95 (0.72–1.26)	0.723	73	76	0.82 (0.55–1.21)	0.313
ADH1C	4147542	283	250	222	185	1.06 (0.80–1.39)	0.687	45	44	0.90 (0.56–1.43)	0.641
ALDH2	4646778	326	284	191	177	0.91 (0.69–1.21)	0.522	39	24	1.55 (0.88–2.73)	0.131
	671	470	402	79	80	0.85 (0.59–1.22)	0.377	12	4	2.55 (0.76–8.53)	0.129
GSTP1	612020	477	412	82	72	1.06 (0.73–1.52)	0.766	3	6	0.32 (0.08–1.38)	0.126
	1695	307	262	219	196	0.91 (0.70–1.19)	0.492	41	31	1.05 (0.62–1.78)	0.851
NAT1	7845127	277	252	229	199	1.03 (0.79–1.35)	0.826	59	39	1.36 (0.85–2.18)	0.196
NAT2	1041983	167	128	263	245	0.82 (0.60–1.11)	0.191	116	107	0.87 (0.60–1.26)	0.462
	1799930	249	214	231	232	0.82 (0.62–1.08)	0.151	72	37	1.56 (0.99–2.47)	0.058
CYP2E1	2249695	179	162	274	233	1.07 (0.80–1.44)	0.639	96	78	1.05 (0.71–1.56)	0.806
	2031920	400	340	135	115	0.92 (0.68–1.25)	0.595	11	18	0.48 (0.21–1.09)	0.079
DRD2	7117915	254	196	246	223	0.92 (0.70-1.21)	0.543	56	65	0.67 (0.44–1.02)	0.064
	10891556	367	336	174	138	1.21 (0.91–1.61)	0.193	18	13	1.32 (0.62–2.82)	0.476
DRD3	2087017	167	141	276	263	0.84 (0.62–1.14)	0.260	124	87	1.15 (0.79–1.67)	0.465
	167770	315	307	219	150	1.36 (1.03–1.80)	0.029	26	23	0.92 (0.49–1.69)	0.778
SLC6A4	140701	310	287	219	172	1.18 (0.90–1.55)	0.231	38	31	1.33 (0.77–2.29)	0.313

[a] OR adjusted by age (<30, 30–45, 45–60, 60<), BMI (<20, 20–25, 25–30, 30<), smoking, pregnancy and breast feeding, family history of breast cancer, education, and menopausal status

MTHFR is a critical gene in the one-carbon metabolism pathway. Two non-synonymous missense SNPs, C677T (Ala222Val, rs1801133) and A1298C (Glu429Ala, rs1801131), in the coding region were extensively studied. However, the previous association studies on the *MTHFR* polymorphism and breast cancer risk showed inconsistent results. Several studies have reported that the *MTHFR* C677T variants were associated with an increased risk of breast cancer in pre-menopausal women [16, 17] or in those with bilateral breast cancer or combined breast and ovarian cancers [18], but some others showed no association between *MTHFR* C677T and breast cancer [19, 20]. In addition, Sharp et al. [21] reported that *MTHFR* 1298CC genotype and compound heterozygosity (677CT and 1298AC) were associated with a reduced risk of developing breast cancer. In this study, our finding did not support a role of *MTHFR* C677T or *MTHFR* A1298C in modifying breast cancer risk in Thai women.

The 5-methyltetrahydrofolate-homocysteine *S*-methyltransferase (MTR; also called methionine synthase), which is essential for maintaining adequate intracellular folate pools, catalyzes the remethylation of homocysteine to methionine and has influence on DNA methylation as well as on nucleic acid synthesis [22]. Vitamin B12 is a cofactor in this methylation process. MTR is maintained in its active from by methionine synthesis reductase (MTRR). In this study, the homozygote carriers of *MTR* (rs1770449 and rs105099) SNPs were associated with breast cancer with OR = 2.21 (95% CI 1.18–4.16) and OR = 2.24 (95% CI 1.19–4.22), respectively. In addition, the A allele of MTRR (rs162049) seems to be associated with breast cancer among postmenopausal women (OR 1.61, 95% CI 1.07–

Table 4 Association between folate and alcohol metabolism genes and breast cancer stratified by menopausal status

Gene name	Premenopausal women							
	Homozygotes of major allele		Heterozygotes			Homozygotes of minor allele		
	Ca	Co	Ca	Co	OR[a] (95% CI)	Ca	Co	OR[a] (95% CI)
MTRR (rs162049)	69	39	119	89	0.79 (0.48–1.32) $P = 0.372$	51	46	0.74 (0.41–1.35) $P = 0.328$
NAT2 (rs1799930)	109	82	89	83	0.82 (0.53–1.26) $P = 0.370$	35	9	2.70 (1.20–6.07) $P = 0.017$
DRD2 (rs10891556)	147	122	83	46	1.62 (1.03–2.56) $P = 0.038$	6	6	0.94 (0.28–3.16) $P = 0.925$
DRD3 (rs167770)	142	106	80	54	1.13 (0.72–1.76) $P = 0.593$	14	13	0.75 (0.32–1.74) $P = 0.499$
SLC6A4 (rs140701)	128	109	97	56	1.56 (1.01–2.41) $P = 0.046$	15	11	1.13 (0.47–2.74) $P = 0.785$
Gene name	Postmenopausal women							
	Homozygotes of major allele		Heterozygotes			Homozygotes of minor allele		
	Ca	Co	Ca	Co	OR[a] (95% CI)	Ca	Co	OR[a] (95% CI)
MTRR (rs162049)	71	91	166	150	1.61 (1.07–2.44) $P = 0.024$	82	72	1.43 (0.14–2.31) $P = 0.889$
NAT2 (rs1799930)	138	132	141	148	0.81 (0.56–1.16) $P = 0.240$	36	28	1.10 (0.76–1.96) $P = 0.612$
DRD2 (rs10891556)	216	213	91	92	0.98 (0.67–1.43) $P = 0.912$	12	7	1.50 (0.44–4.14) $P = 0.543$
DRD3 (rs167770)	171	200	137	96	1.59 (1.11–2.28) $P = 0.011$	12	10	1.16 (0.75–2.89) $P = 0.468$
SLC6A4 (rs140701)	180	178	120	115	0.93 (0.65–1.33) $P = 0.687$	23	20	1.46 (0.30–2.96) $P = 0.720$

[a] OR adjusted by age (<30, 30–45, 45–60, 60<), BMI (<20, 20–25, 25–30, 30<), smoking, pregnancy and breast feeding, family history of breast cancer, and education

2.44). However, these SNPs have never been reported with breast cancer before and their functional effect on enzymatic activities remain unknown.

Alcohol drinking is among the non-hormonal risk factors for breast cancer (although there may be an indirect relationship). Genetic susceptibility for ethanol metabolism can affect breast cancer, and several genes are known to be involved in this complex pathway. Our study did not find any affect of *ADH1C*, *ALDH2*, *CYP2E1*, *GSTP1*, and *NAT1* polymorphisms on breast cancer risk. However, the homozygotes of minor allele of *NAT2* (rs1799930) SNP was associated with an increased risk of breast cancer among premenopausal (OR 2.70, 95% CI 1.20–6.07), although the number of the subjects were limited. Lu et al. [23] showed an interaction effect in bladder cancer between *NAT2* polymorphism and alcohol drinking. In addition, Rodrigo et al. [24] reported that NAT2 activity may be a factor that determines the risk of developing alcoholic liver disease. In this study, the genotype distribution of the *NAT2* (rs1799930) polymorphism departed from Hardy–Weinberg Equilibrium, and the interpretation of the result should be warranted.

Furthermore, many studies suggested that the intensity of drinking (drink per day) has more effect on risk for breast cancer than recent alcohol or duration of drinking [25, 26]. We, therefore, investigated the SNPs of *DRD2*, *DRD3*, and *SLC6A4*, which are implicated in drinking behavior. In animal studies, alcohol can stimulate dopaminergic neurons in the ventral tegmental area [27, 28], and the density of dopamine D2 receptors in the limbic system is lower in alcohol-preferring rats than in non-preferring rats [29, 30]. Likewise, the number of striatal dopamine D2 receptors is less in alcohol-preferring humans than in healthy control subjects. The A1 polymorphism of *DRD2 Taq*I A loci has been considered as a risk factor for alcohol dependence [31, 32], but the association between alcoholism and the *DRD2* gene remains equivocal in many studies [33–35]. In our study, an

Table 5 Association of selected SNPs and breast cancer risk by alcohol, BMI, and physical activity

Variable	*MTR* (rs1770449) (case/control)		OR[a] (95% CI)		*MTR* (rs1050993) (case/control)	
	AA	AG/GG	AA	AG/GG	GG	GA/GG
Alcohol						
Yes	27/14	18/12	1	1.198 (0.28–5.13)	27/15	17/12
No	203/178	318/282	1	1.053 (0.80–1.38)	317/284	203/178
P for interaction				$P = 0.64$		
BMI (kg/m^2)						
<25	217/224	142/146	1	1.033 (0.75–1.41)	216/228	141/145
≥25	128/72	79/44	1	1.042 (0.63–1.72)	128/71	79/45
P for interaction				$P = 0.97$		
OC use						
Yes	157/121	99/76	1	1.054 (0.71–1.56)	157/123	99/76
No	187/171	122/114	1	1.007 (0.71–1.44)	186/172	121/114
P for interaction				$P = 0.75$		

Variable	OR (95% CI)		*DRD3* (rs167770) (case/contol)		OR (95% CI)	
	GG	GA/GG	AA	AG/GG	AA	AG/GG
Alcohol						
Yes	1	1.339 (0.32–5.53)	27/16	17/11	1	0.518 (0.12–2.23)
No	1	1.059 (0.81–1.39)	288/291	228/162		1.355 (1.03–1.78)
P for interaction		$P = 0.69$				$P = 0.24$
BMI (kg/m^2)						
<25	1	1.063 (0.78–1.45)	210/235	143/129	1	1.204 (0.88–1.65)
≥25	1	1.002 (0.61–1.65)	105/72	102/44	1	0.253 (0.06–1.02)
P for interaction		$P = 0.86$				$P = 0.39$
OC use						
Yes	1	1.026 (0.72–1.47)	141/127	115/67	1	1.111 (0.77–1.59)
No	1	1.054 (0.71–1.57)	174/178	129/104	1	1.56 (1.05–2.33)
P for interaction		$P = 0.81$				$P = 0.21$

[a] OR adjusted by age(<30, 30–45, 45–60, 60<), BMI (<20, 20–25, 25–30, 30<), smoking, menopausal status, family history of breast cancer, and education

Table 6 Association of *DRD3* and *MTRR* polymorphisms with the risk of breast cancer

Genotype	*DRD3* (AA)				*DRD3*(AG/GG)				*P* for interaction
	Cases	Controls	OR[a]	95% CI	Cases	Controls	OR	95% CI	
MTR (rs1770449)									
AA	184	188	1		155	102	1		
AG	108	103	1.07	0.74–1.53	74	67	0.72	0.46–1.13	0.096
GG	20	14	1.93	0.89–4.18	16	4	3.78	1.04–13.6	0.25
MTR (rs1050993)									
GG	183	191	1		155	102	1		
GA	108	102	1.09	0.76–1.56	74	66	0.73	0.46–1.15	0.10
AA	20	14	1.96	0.91–4.24	16	4	3.77	1.04–13.6	0.26

[a] OR adjusted by age (<30, 30–45, 45–60, 60<), BMI (<20, 20–25, 25–30, 30<), smoking, menopausal status, family history of breast cancer, and education

association was suggested between the *DRD3* SNP (rs167770) in an overall or in postmenopausal breast cancer (OR 1.36, 95% CI 1.03–1.80 and OR 1.59, 95% CI 1.11–2.28, respectively). The *DRD2* (rs10891556) polymorphism was associated with an increased risk among premenopausal women (OR 1.62, 95% CI 1.03–2.56). The propensity for severe drinking has been also hypothesized to be regulated by differential expression of serotonin transporter gene *SLC6A4* [36]. Our result suggested that *SCL6A4* (rs140701) was associated with an increased risk of breast cancer among premenopausal (OR 1.56, 95% CI 1.01–2.41). As the alcohol consumption was relatively low among Thai women in our study, it may have reduced the ability to detect a modifying effect of ethanol on the association of these genes with breast cancer. The current knowledge on genetic polymorphisms related to drinking behavior is far from sufficient. The possible associations between the genetic polymorphisms implicated in drinking behavior and breast cancer risk need to be confirmed in a population with a higher prevalence of alcohol drinking than Thai women.

Certain limitations of this study should be noted. First, data were not available on detailed dietary intake of folate, plasma or erythrocyte folate levels and its precursors or metabolites such as homocysteine, limiting further examination of the gene–nutrient interactions in breast carcinogenesis. In this study, a food frequency questionnaire was administered by trained interviewers to assess dietary and alcohol intake. Unfortunately, we could not calculate the total folate in individual intake due to lack of the standard food composition in Thailand. Second, like most other case–control studies, this study may suffer from recall bias. Third, the statistical power of our study was limited in the stratified analyses because of the small sample size of the subgroups. For instance, if the simple but conservative Bonferroni correction for multiple testing is applied to our data set, none of the SNPs remained statistically significant. Although this study reports a systematic survey of genetic polymorphisms on the folate and alcohol metabolic pathways on breast cancer in Thailand for the first time, it is primarily for a hypothesis generation, and the findings need to be validated in further studies with larger sample size or in meta-analyses, which is also aimed in our laboratory for years to come.

In conclusion, our study has provided some new evidence that either folate metabolism genes or alcohol metabolism and behavior gene polymorphisms may contributes to the etiology of breast cancer among Thai women. Studies should be extended to cover SNPs of other important one-carbon or alcohol metabolism genes, and ascertainment of high quality folate intake information is expected to further elucidate gene–gene and gene–environment interactions in susceptibility of breast cancer.

Acknowledgments We express our sincere thanks to all patients, recruited control subjects, doctors, nurses, and paramedical personnel of National Cancer Institute, Thailand for their kind participation. This work was supported by the program for promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NiBio 05-41), Japan. S. Sangrajrang is an awardee of a fellowship from Foundation for Promotion of Cancer Research.

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