Short Communication

Association of Thymidylate Synthase Gene with Endometrial Cancer Risk in a Chinese Population

Wang-Hong Xu,[1,2] Ji-Rong Long,[3] Wei Zheng,[3] Zhi-Xian Ruan,[1] Qiuyin Cai,[3] Jia-Rong Cheng,[1] Gen-Ming Zhao,[2] Yong-Bing Xiang,[1] and Xiao-Ou Shu[3]

[1]Department of Epidemiology, Cancer Institute of Shanghai Jiao Tong University, Shanghai Cancer Institute; [2]Department of Epidemiology, School of Public Health, Fudan University, Shanghai, People's Republic of China and [3]Vanderbilt Epidemiology Center, Department of Medicine and Vanderbilt-Ingram Cancer Center, Nashville, Tennessee

Abstract

We comprehensively evaluated genetic variants in the thymidylate synthase (*TYMS*) gene in association with endometrial cancer risk in a population-based case-control study of 1,199 incident endometrial cancer cases and 1,212 age frequency-matched population controls. Exposure information was obtained via in-person interview, and DNA samples (blood or buccal cell) were collected. Genotyping of 11 haplotype-tagging single nucleotide polymorphisms (SNP) for the *TYMS* gene plus the 5-kb flanking regions was done for 1,028 cases and 1,003 controls by using the Affymetrix MegAllele Targeted Genotyping System. Of 11 haplotype-tagging SNPs identified, 7 that are located in flanking regions of the *TYMS* gene are also in the *ENOSF1* (*rTS*) gene. The SNP rs3819102, located in the 3′-flanking region of the *TYMS* gene and in an intron of the *ENOSF1* gene, was associated with risk of endometrial cancer. The odds ratio (95% confidence interval) for the CC genotype was 1.5 (1.0-2.2) compared with the TT genotype. Haplotype TTG in block 2 of the *TYMS* gene, which includes SNPs rs10502289, rs2298583, and rs2298581 (located in introns of the *ENOSF1* gene), was associated with a marginally significant decrease in risk of endometrial cancer under the dominant model (odds ratio, 0.8; 95% confidence interval, 0.6-1.0). This study suggests that genetic polymorphisms in the *TYMS* or *ENOSF1* genes may play a role in the development of endometrial cancer among Chinese women. (Cancer Epidemiol Biomarkers Prev 2009;18(2):579–84)

Introduction

We have reported previously a significant inverse association between dietary folate intake, the major source of the dietary methyl groups that are involved in DNA methylation, synthesis, and repair (1), and risk of endometrial cancer (2). This association was modified by *MTHFR* polymorphisms, suggesting an important role for folate in this disease. Folate intake and *MTHFR* polymorphisms have also been associated with cancers of the breast and colon (3, 4).

Thymidylate synthase (TYMS), encoded by the *TYMS* gene, is another enzyme important for folate synthesis (4). TYMS catalyzes the transformation of dUMP to dTMP and is the only *de novo* source of thymidylate used for DNA biosynthesis (4), and TYMS competes with MTHFR for the limited supplies of folate present in the body and that are required for remethylation of homocysteine. Altered TYMS activity may change the availability of folate and homocysteine (5). TYMS also functions as a RNA-binding protein for translational repression of its own and other downstream mRNAs (6, 7) and may induce dysregulation in DNA biosynthesis, DNA repair, and cell cycle progression.

TYMS polymorphisms, including a 28-bp tandem repeat variant in the enhancer region and a 6-bp deletion in the 3′-untranslated region, have been linked to the risk of colorectal (4, 8-10) and breast (11, 12) cancers presumably because they alter the activity of TYMS (13-15). It is plausible that *TYMS* polymorphisms also play a role in the development of endometrial cancer, a hormone-dependent disease like breast cancer and the most common extracolonic malignancy of the hereditary nonpolyposis colorectal cancers. This hypothesis, to our knowledge, has not been evaluated previously.

In this study, we evaluated whether genetic polymorphisms in the *TYMS* gene confer susceptibility to endometrial cancer by using a haplotype-tagging single nucleotide polymorphism (htSNP) approach using data from the Shanghai Endometrial Cancer Study, a large population-based, case-control study conducted in urban Shanghai, China.

Materials and Methods

As described previously (2), of the 1,449 newly diagnosed endometrial cancer cases at ages 30 to 69 years

Received 9/8/08; revised 11/6/08; accepted 11/13/08; published OnlineFirst 02/03/2009.

Grant support: National Cancer Institute/USPHS grant R01CA92585. The Vanderbilt Microarray Shared Resource is supported by Vanderbilt-Ingram Cancer Center grant P30 CA68485, Vanderbilt Diabetes Research and Training Center grant P60 DK20593, Vanderbilt Digestive Disease Center grant P30 DK58404, and Vanderbilt Vision Center grant P30 EY08126.

Requests for reprints: Xiao-Ou Shu, Vanderbilt Epidemiology Center, Institute for Medicine and Public Health, Vanderbilt University Medical Center, 2525 West End Avenue, Suite 600, Nashville, TN 37203-1738. Phone: 615-936-0713; Fax: 615-936-8291. E-mail: Xiao-ou.shu@vanderbilt.edu

doi:10.1158/1055-9965.EPI-08-0831

who were identified between 1997 and 2003 through the population-based Shanghai Cancer Registry, 1,199 (82.7%) participated in the study. Controls were randomly selected from the general population of urban Shanghai using the Shanghai Resident Registry and frequency matched to cases on age distribution. Women with a history of any cancer or hysterectomy were not eligible. Of the 1,629 eligible women contacted, 1,212 (74.4%) participated in the study. The study protocols were approved by the institutional review boards of all participating institutes, and all participants provided written informed consent.

Detailed information on demographic, reproductive, medical history, and lifestyle factors was collected via an in-person interview. Body weight, height, and circumferences of the waist and hips were measured by trained interviewers according to a standardized protocol at the time of interview. Menopause was defined as the cessation of the menstrual period for at least 12 months before diagnosis for cases and interview for controls, excluding those lapses caused by pregnancy, breast-feeding, or estrogen hormone use. Body mass index [weight (kg)/height (m^2)] and waist-to-hip circumference ratio were calculated using measured anthropometrics.

DNA samples from 1,037 cases (86.5%; 850 blood and 187 buccal cell) and 1,020 controls (84.2%; 834 blood and 186 buccal cell) were included in the genotyping study. SNP selection was completed in December 2005. As listed in Appendix 1, 11 htSNPs were selected by searching Han Chinese data from the HapMap project[4] using the Tagger program (16) according to following criteria: (*a*) SNPs were located in the TYMS gene or within the 5-kb region flanking the gene, (*b*) had a minor allele frequency ≥ 0.05, and (*c*) the other unselected SNPs could be captured by one of the tagging SNPs with a linkage disequilibrium (LD) $r^2 \geq 0.90$. It is worth noting that 7 htSNPs in the TYMS flanking region are located in introns of the ENOSF1 gene.

The SNPs were genotyped using the Affymetrix MegAllele Targeted Genotyping System with the Molecular Inversion Probe method (17) as a part of large-scale genotyping efforts that included 1,737 SNPs. Genotyping was conducted at the Vanderbilt Microarray Shared Resource following the manufacturer's protocol. The laboratory staff remained blinded to the case-control status and identity of all samples. The consistency rate for 39 blinded duplicated quality-control samples and 12 HapMap DNA samples in the genotyping was >97.4%. The genotyping of *TYMS* SNPs was highly successful, with call rates of 99.5% to 100% (median, 99.95%). Consequently, *TYMS* genotyping data were obtained from 1,028 cases and 1,003 controls, with a success rate of 99.1% and 98.3%, respectively.

χ^2 statistics and the *t* test were used to evaluate case-control differences in the distribution of risk factors and genotypes of the *TYMS* gene. Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI). Interactive effects were evaluated in logistic regression analyses using the likelihood ratio test by comparing the model including the main effects only with that including both the main effects and the interaction terms. LD between polymorphisms was assessed by HaploView software (18), and haplotype blocks were defined using the methods of Gabriel et al. (19). Haplotype analyses were conducted using HapStat software (20) and logistic regression models. All statistical tests were based on two-tailed probability.

Results

The case-control differences on demographic and non-genetic risk factors for endometrial cancer have been reported previously (2). No appreciable differences were seen for the subgroup of participants included in the current analysis (data not shown) and the entire study population.

The distributions of 11 *TYMS* htSNPs were all consistent with Hardy-Weinberg equilibrium among controls. rs3819102, a SNP located in the 3′-flanking region of the *TYMS* gene and in an intron of the *ENOSF1* gene, was associated with the risk of endometrial cancer. Compared with the TT genotype, genotype CC was associated with increased risk (OR, 1.5; 95% CI, 1.0-2.2); the OR (95% CI) per allele was 1.1 (1.0-1.3; $P_{trend} = 0.14$). No significant association was observed for the other 10 htSNPs with cancer risk (Table 1).

We further evaluated the modifying effect of menopausal status in gene-disease associations and found that the C allele at rs3819102 was associated with an increased risk of endometrial cancer among postmenopausal but not premenopausal women [OR (95% CI) was 1.1 (0.9-1.5) for the CT genotype and 1.7 (1.1-2.8) for the CC genotype compared with the TT genotype; $P_{trend} = 0.03$]. However, the test for interaction was not significant ($P_{interaction} = 0.24$; Table 1). No other estrogen exposure factors such as years of menstruation, oral contraceptive use, body mass index, or waist-to-hip circumference ratio interacted with SNP rs3819102 in cancer development (data not shown). We also did not observe a significant interactive effect for any *TYMS* htSNPs with folate intake (high/low by 75% quartile intake), vitamin supplement use (never/ever), or *MTHFR* polymorphisms (rs1801133, rs1801131, and rs2274976; data not shown).

Two haplotype blocks were observed in the TYMS gene. Five SNPs, one in exon 3 (rs3786362), one in intron 4 (rs2853532), and the other three in the 3′-flanking region (rs3744962, rs11081251, and rs9948583), comprised LD block 1. Three other SNPs in the 3′-flanking region (rs10502289, rs2298583, and rs2298581) comprised LD block 2. Five common haplotypes (frequency >5%) for the five polymorphic sites in block 1 were reconstructed, and four haplotypes for the three polymorphic sites in block 2 were estimated. The frequencies of haplotypes in blocks 1 and 2 did not differ significantly between cases and controls. None of the haplotypes in block 1 was significantly associated with endometrial cancer risk (Table 2). Haplotype TTG in block 2 was associated with a borderline significant reduction in risk under the dominant model (OR, 0.8; 95% CI, 0.6-1.0; $P = 0.07$) and with an OR (95% CI) of 0.8 (0.7-1.1) compared with the most common allele haplotype TCG. Further analysis did not reveal any significant interaction between haplotypes and menopausal status or other estrogen exposure factors (data not shown).

[4] http://www.hapmap.org

Table 1. Association of the htSNPs in the *TYMS* gene with endometrial cancer risk, Shanghai Endometrial Cancer Study, 1997-2003

SNPs at *TYMS* gene	Minor allele frequency in controls	All subjects			OR per allele	P_{trend}	Premenopausal women			OR per allele	P_{trend}	Postmenopausal women			OR per allele	P_{trend}
		Cases (%)	Controls (%)	OR (95% CI)			Cases (%)	Controls (%)	OR (95% CI)			Cases (%)	Controls (%)	OR (95% CI)		
rs502396	0.280	1,027	1,003				444	386				583	617			
CC		555 (54.0)	520 (51.8)	1.0			256 (57.7)	206 (53.4)	1.0			299 (51.3)	314 (50.9)	1.0		
CT		390 (38.0)	406 (40.5)	0.9 (0.7-1.1)			156 (35.1)	151 (39.1)	0.8 (0.6-1.1)			234 (40.1)	255 (41.3)	1.0 (0.8-1.2)		
TT		82 (8.0)	77 (7.7)	1.0 (0.7-1.4)	1.0 (0.8-1.1)	0.50	32 (7.2)	29 (7.5)	1.0 (0.6-1.6)	0.9 (0.7-1.1)	0.39	50 (8.6)	48 (7.8)	1.1 (0.7-1.7)	1.0 (0.8-1.2)	0.89
rs2244500	0.310	1,028	1,003				444	386				584	617			
CC		509 (49.5)	469 (46.8)	1.0			237 (53.4)	192 (49.7)	1.0			272 (46.6)	277 (44.9)	1.0		
CT		410 (39.9)	444 (44.3)	0.9 (0.7-1.0)			163 (36.7)	162 (42.0)	0.8 (0.6-1.1)			247 (42.3)	282 (45.7)	0.9 (0.7-1.1)		
TT		109 (10.6)	90 (9.0)	1.1 (0.8-1.5)	1.0 (0.9-1.1)	0.70	44 (9.9)	32 (8.3)	1.2 (0.7-1.9)	1.0 (0.8-1.2)	0.75	65 (11.1)	58 (9.4)	1.1 (0.8-1.7)	1.0 (0.8-1.2)	0.98
rs3786362	0.188	1,031	1,015				443	386				579	614			
TT		683 (66.8)	659 (65.9)	1.0			278 (62.8)	245 (63.5)	1.0			405 (70.0)	414 (67.4)	1.0		
CT		305 (29.8)	306 (30.6)	1.0 (0.8-1.2)			151 (34.1)	126 (32.6)	1.0 (0.8-1.4)			154 (26.6)	180 (29.3)	0.9 (0.7-1.1)		
CC		34 (3.3)	35 (3.5)	0.9 (0.6-1.5)	1.0 (0.8-1.1)	0.65	14 (3.2)	15 (3.9)	0.9 (0.4-2.0)	1.0 (0.8-1.3)	0.94	20 (3.5)	20 (3.3)	1.0 (0.5-1.9)	0.9 (0.7-1.1)	0.44
rs2853532	0.317	1,027	1,003				443	386				584	617			
TT		499 (48.6)	463 (46.2)	1.0			231 (52.1)	188 (48.7)	1.0			268 (45.9)	275 (44.6)	1.0		
CT		417 (40.6)	444 (44.3)	0.9 (0.7-1.0)			167 (37.7)	165 (42.8)	0.8 (0.6-1.1)			250 (42.8)	279 (45.2)	0.9 (0.7-1.2)		
CC		111 (10.8)	96 (9.6)	1.1 (0.8-1.4)	1.0 (0.9-1.1)	0.69	45 (10.2)	33 (8.6)	1.2 (0.7-1.9)	1.0 (0.8-1.2)	0.79	66 (11.3)	63 (10.2)	1.1 (0.7-1.6)	1.0 (0.8-1.2)	0.96
rs3744962	0.158	1,027	1,002				444	386				583	616			
TT		741 (72.2)	713 (71.2)	1.0			331 (74.6)	289 (74.9)	1.0			410 (70.3)	424 (68.8)	1.0		
CT		257 (25.0)	260 (26.0)	1.0 (0.8-1.2)			102 (23.0)	89 (23.1)	1.0 (0.7-1.4)			155 (26.6)	171 (27.8)	0.9 (0.7-1.2)		
CC		29 (2.8)	29 (2.9)	1.0 (0.6-1.6)	1.0 (0.8-1.1)	0.65	11 (2.5)	8 (2.1)	1.2 (0.5-3.0)	1.0 (0.8-1.3)	0.91	18 (3.1)	21 (3.4)	0.9 (0.5-1.7)	0.9 (0.8-1.2)	0.57
rs11081251	0.309	1,028	1,003				444	386				584	617			
CC		512 (49.8)	477 (47.6)	1.0			239 (53.8)	194 (50.3)	1.0			273 (46.8)	283 (45.9)	1.0		
AC		409 (39.8)	432 (43.1)	0.9 (0.7-1.1)			160 (36.0)	160 (41.5)	0.8 (0.6-1.1)			249 (42.6)	272 (44.1)	1.0 (0.7-1.2)		
AA		107 (10.4)	94 (9.4)	1.1 (0.8-1.4)	1.0 (0.9-1.1)	0.68	45 (10.1)	32 (8.3)	1.2 (0.7-2.0)	1.0 (0.8-1.2)	0.80	62 (10.6)	62 (10.1)	1.0 (0.7-1.5)	1.0 (0.8-1.2)	0.95
rs9948583	0.320	1,027	1,001				443	386				584	615			
TT		485 (47.2)	463 (46.3)	1.0			230 (51.9)	191 (49.5)	1.0			255 (43.7)	272 (44.2)	1.0		
CT		427 (41.6)	436 (43.6)	0.9 (0.8-1.1)			166 (37.5)	161 (41.7)	0.9 (0.6-1.1)			261 (44.7)	275 (44.7)	1.0 (0.8-1.3)		
CC		115 (11.2)	102 (10.2)	1.1 (0.8-1.4)	1.0 (0.9-1.1)	0.99	47 (10.6)	34 (8.8)	1.2 (0.7-1.9)	1.0 (0.8-1.2)	0.97	68 (11.6)	68 (11.1)	1.1 (0.7-1.6)	1.0 (0.9-1.2)	0.76
rs3819102	0.237	1,027	1,001				444	386				583	615			
TT		574 (55.9)	576 (57.5)	1.0			257 (57.9)	213 (55.2)	1.0			317 (54.4)	363 (59.0)	1.0		
CT		379 (36.9)	376 (37.6)	1.0 (0.8-1.2)			158 (35.6)	154 (39.9)	0.8 (0.6-1.1)			221 (37.9)	222 (36.1)	1.1 (0.9-1.5)		
CC		74 (7.2)	49 (4.9)	1.5 (1.0-2.2)	1.1 (1.0-1.3)	0.14	29 (6.5)	19 (4.9)	1.3 (0.7-2.3)	1.0 (0.8-1.2)	0.72	45 (7.7)	30 (4.9)	1.7 (1.1-2.8)	1.2 (1.0-1.5)	0.03
rs10502289	0.154	1,025	1,001				443	386				582	615			
TT		726 (70.8)	721 (72.0)	1.0			307 (69.3)	278 (72.0)	1.0			419 (72.0)	443 (72.0)	1.0		
AT		271 (26.4)	255 (25.5)	1.1 (0.9-1.3)			121 (27.3)	99 (25.7)	1.1 (0.8-1.5)			150 (25.8)	156 (25.4)	1.0 (0.8-1.3)		
AA		28 (2.7)	25 (2.5)	1.1 (0.6-1.9)	1.1 (0.9-1.2)	0.53	15 (3.4)	9 (2.3)	1.6 (0.6-3.5)	1.1 (0.9-1.5)	0.39	13 (2.2)	16 (2.6)	0.9 (0.4-1.8)	1.0 (0.8-1.2)	0.90
rs2298583	0.471	1,027	1,002				443	386				584	616			
CC		306 (29.8)	282 (28.1)	1.0			115 (26.0)	99 (25.7)	1.0			191 (32.7)	183 (29.7)	1.0		
CT		504 (49.1)	496 (49.5)	0.9 (0.8-1.1)			226 (51.0)	193 (50.0)	1.0 (0.7-1.3)			278 (47.6)	303 (49.2)	0.9 (0.7-1.1)		
TT		217 (21.1)	224 (22.4)	0.9 (0.7-1.1)	0.9 (0.8-1.1)	0.36	102 (23.0)	94 (24.4)	0.9 (0.6-1.4)	1.0 (0.8-1.2)	0.66	115 (19.7)	130 (21.1)	0.8 (0.6-1.2)	0.9 (0.8-1.1)	0.27
rs2298581	0.389	1,024	1,003				441	386				583	617			
GG		386 (37.7)	375 (37.4)	1.0			149 (33.8)	139 (36.0)	1.0			237 (40.7)	236 (38.3)	1.0		
CG		485 (47.4)	478 (47.7)	1.0 (0.8-1.2)			218 (49.4)	186 (48.2)	1.1 (0.8-1.4)			267 (45.8)	292 (47.3)	0.9 (0.7-1.2)		
CC		153 (14.9)	150 (15.0)	1.0 (0.8-1.3)	1.0 (0.9-1.1)	0.92	74 (16.8)	61 (15.8)	1.1 (0.8-1.7)	1.1 (0.9-1.3)	0.54	79 (13.6)	89 (14.4)	0.9 (0.6-1.3)	0.9 (0.8-1.1)	0.39

NOTE: OR: adjusted for age. Additional adjustment for education, menopausal status, diabetes, alcohol consumption, physical activity, and body mass index did not change the results materially.
$P_{interaction}$ test was 0.24 between menopausal status and rs3819102 genotypes.

Discussion

In this population-based, case-control study, rs3819102, a htSNP located in the 3′-flanking region of the *TYMS* gene and an intron of the *ENOSF1* gene, was found to be associated with an increased risk of endometrial cancer. An association was also indicated for haplotype TTG at block 2 of the *TYMS* gene under the dominant model. To our knowledge, this is the first study that has evaluated the role of the *TYMS* gene in endometrial cancer risk using a comprehensive approach.

The *TYMS* gene is located at 18p11.32. Two polymorphic sites in this gene, a series of 28-bp tandem repeats in the enhancer region and a 6-bp deletion (rs11280056) in the 3′-untranslated region, have been shown to be involved in regulation of *TYMS* mRNA expression (13, 14) and linked to alteration of TYMS activity (13-15). These two polymorphisms cause altered levels of folate and homocysteine (5, 14) and imbalances in the deoxynucleotide pool in the cell (21), which have been linked to DNA damage, altered DNA replication, and impaired mechanisms of DNA repair experimentally (22-24). Epidemiologic studies have also suggested that these two functional polymorphisms may be associated with cancers of colon/rectum (8-10), breast (11, 12), esophagus (25), stomach (25-27), head and neck (28), lung (29), and liver (30). No previous studies, however, have investigated the association between the *TYMS* gene and endometrial cancer.

In this study, we used a htSNP approach to investigate the role of the *TYMS* gene in the development of endometrial cancer. Because the two functional polymorphisms mentioned above were not SNPs, they could not be genotyped using the Affymetrix Targeted Genotyping system and thus were not included in the present study. In a recent Japanese study (31), the 28-bp tandem repeat polymorphism did not show any distinct association with other detected upstream and downstream SNPs. However, based on HapMap data, we found that rs11280056 is in perfect LD ($r^2 = 1$) with SNPs rs2853536, rs2853537, rs1059394, and rs699517, which are in strong LD ($r^2 > 0.8$) with rs11081251, a SNP included in our study. Thus, it is possible that the association of the insertion/deletion variant rs11280056 with endometrial cancer is captured by SNP rs11081251 in the current study. We did not find rs11081251 to be associated with endometrial cancer risk. Instead, our results suggest that rs3819102 and the haplotype TTG in block 2 of the gene may be associated with endometrial cancer. It is noteworthy that none of the 3 SNPs forming the informative haplotype were individually related to disease risk, suggesting the possible presence of gene-gene interaction.

In this study, 7 SNPs located in the *TYMS* flanking regions are also in the *ENOSF1* gene. The *ENOSF1* gene was originally identified as a naturally occurring antisense transcript to the human *TYMS* gene (32) and codes for two proteins (rTSα and rTSβ) through alternative RNA splicing (32, 33). The function of the *ENOSF1* gene appears primarily to regulate the expression of the *TYMS* locus both via the antisense transcript and through the encoded proteins (34, 35). Given that SNP rs3819102 and three polymorphic sites in block 2 are also in the introns of the *ENOSF1* gene, it is possible that these polymorphisms may be involved in endometrial carcinogenesis through regulation of *TYMS* gene expression.

Estrogen levels also function as a regulator of *TYMS* expression (36), so it is plausible that menopausal status or other estrogen-related factors may interact with these genetic polymorphisms. In our study, a possible modifying effect of menopausal status was suggested, but tests for multiplicative interaction were not significant.

Strengths of this study include the population-based design, high participation rate, homogeneous ethnic background (>98% Han Chinese), low hormone replacement therapy use, and low frequency of hysterectomy (5.1%) in the study population. The application of the htSNP approach in SNP selection made it possible to systematically evaluate the genetic markers of the *TYMS* gene. However, the sample size was not sufficiently large for testing moderate interactions. Although our study has adequate power (>85%) to detect a moderate gene effect (minimum detectable OR, 1.35), it is underpowered to detect small gene or interactive effects. Chance

Table 2. Association of *TYMS* haplotypes with the risk of endometrial cancer

TYMS	Cases (%), n = 1,028	Controls (%), n = 1,003	OR (95% CI)*		OR (95% CI)†
			Dominant model	Recessive model	Additive model
Block1‡					
TTTCT	48.8	48.4	1.0 (0.8-1.3)	1.0 (0.8-1.2)	1.0 (reference)
CTTCT	18.3	18.8	1.0 (0.8-1.2)	1.0 (0.7-1.4)	1.0 (0.8-1.1)
TCCAC	15.3	15.9	0.9 (0.8-1.1)	1.2 (0.8-1.8)	0.9 (0.8-1.1)
TCTAC	15.0	15.0	1.0 (0.8-1.2)	1.1 (0.7-1.7)	1.0 (0.8-1.2)
Others	2.6	1.9			1.4 (0.9-2.1)
Block 2§					
TCG	54.3	52.7	1.0 (0.8-1.2)	1.1 (0.9-1.3)	1.0 (reference)
TTC	22.7	23.6	1.0 (0.8-1.2)	0.9 (0.8-1.3)	0.9 (0.8-1.1)
ATC	15.9	15.2	0.9 (0.8-1.1)	1.2 (0.8-1.7)	1.0 (0.8-1.2)
TTG	7.0	8.4	0.8 (0.6-1.0)	1.0 (0.4-2.3)	0.8 (0.7-1.1)
Others	0.1	0.1			0.6 (0.1-3.8)

*Calculated with HapStat software. Adjusted for age. Additional adjustment for education, menopausal status, diabetes, alcohol consumption, physical activity, and body mass index did not change the results materially.
† Calculated with logistic regression model under additive genetic model.
‡ In the order: rs3786362, rs2853532, rs3744962, rs11081251, and rs9948583.
§ In the order: rs10502289, rs2298583, and rs2298581.

Appendix 1. Primary information for genotyped SNPs of the TYMS gene, Shanghai Endometrial Cancer Study, 1997-2003

SNP	Location	Allele	P_{HWE} for cases	P_{HWE} for controls	Call rate (%)
rs502396	649236, intron 1	C>**T**	0.12	0.90	99.9
rs2244500	651005, intron 2	C>**T**	0.04	0.37	100.0
rs3786362	652247, exon 3, synonymous	**C**>T	0.89	0.85	99.6
rs2853532	660414, intron 4	C>**T**	0.08	0.52	99.9
rs3744962	664320, flanking	**C**>T	0.18	0.19	99.9
rs11081251	664440, flanking	**A**>C	0.05	0.83	100.0
rs9948583	665000, flanking	**C**>T	0.16	0.99	99.8
rs3819102	665307, flanking	**C**>T	0.29	0.17	99.8
rs10502289	666789, flanking	**A**>T	0.61	0.80	99.7
rs2298583	667302, flanking	C>**T**	0.59	0.89	99.9
rs2298581	667931, flanking	**C**>G	0.78	0.63	99.8

Location version: National Center for Biotechnology Information Build 36. Alleles in bold are minor alleles. HWE, Hardy-Weinberg equilibrium.

findings that resulted from multiple comparisons also cannot be excluded.

In summary, we found that SNP rs3819102 and the haplotype TTG in block 2, both located in the 3′-flanking region of the *TYMS* gene and the introns of the *ENOSF1* gene, may be associated with endometrial cancer. Further studies are needed to confirm our findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The study would not have been possible without the support of the study participants and research staff of the Shanghai Endometrial Cancer Study. We thank Dr. Fan Jin for contributions to implementation of the study in Shanghai, Regina Courtney, Dr. Shawn Levy, and the Vanderbilt Microarray Shared Resource for contributions to the genotyping, and Bethanie Hull for assistance in the preparation of this article. All microarray experiments were done at the Vanderbilt Microarray Shared Resource.

References

1. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporaion into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290–5.
2. Xu WH, Shrubsole MJ, Xiang YB, et al. Dietary folate intake, MTHFR genetic polymorphisms and the risk of endometrial cancer among Chinese women. Cancer Epidemiol Biomarker Prev 2007;16:281–7.
3. Lewis SJ, Harbord RM, Harris R, Smith GD. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. J Natl Cancer Inst 2006;98:1607–22.
4. Sharp L, Little J. Human Genome Epidemiology (HuGE) review. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159:423–43.
5. Trinh BN, Ong CN, Coetzee GA, Yu MC, Laird PW. Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. Hum Genet 2002;111:299–302.
6. Chu E, Allegra CJ. The role of thymidylate synthase as an RNA binding protein. BioEssays 1996;18:191–8.
7. Kastanos EK, Zajac-Kaye M, Dennis PA, Allegra CJ. Downregulation of p21/WAF1 expression by thymidylate synthase. Biochem Biophys Res Commun 2001;285:195–200.
8. Ulrich CM, Curtin K, Potter JD, Bigler J, Caan B, Slattery ML. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2509–16.
9. Chen J, Hunter DJ, Stampfer MJ, et al. Polymorphism in the thymidylate synthase promoter enhancer region modifies the risk and survival of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2003;12:958–62.
10. Matsuo K, Ito H, Wakai K, et al. One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. Carcinogenesis 2005;26:2164–71.
11. Xu X, Gammon MD, Zhang H, et al. Polymorphisms of one-carbon-metabolizing genes and risk of breast cancer in a population-based study. Carcinogenesis 2007;28:1504–9.
12. Zhai X, Gao J, Hu Z, et al. Polymorphisms in thymidylate synthase gene and susceptibility to breast cancer in a Chinese population: a case-control analysis. BMC Cancer 2006;6:138.
13. Horie N, Aiba H, Oguro K, Hojo H, Takeishi K. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct 1995;20:191–7.
14. Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol Biomarkers Prev 2000;9:1381–5.
15. Nief N, Le Morvan V, Robert J. Involvement of gene polymorphisms of thymidylate synthase in gene expression, protein activity and anticancer drug cytotoxicity using the NCI-60 panel. Eur J Cancer 2007;43:955–62.
16. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Gene 2005;37:1217–23.
17. Hardenbol P, Yu F, Belmont J, et al. Highly multiplexed molecular inversion probe genotyping: over 10,000 targeted SNPs genotyped in a single tube assay. Genome Res 2005;15:269–75.
18. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
19. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–9.
20. Lin DY, Zeng D, Millikan R. Maximum likelihood estimation of haplotype effects and haplotype-environment interactions in association studies. Genet Epidemiol 2005;29:299–312.
21. James SJ, Basnakian AG, Miller BJ. *In vitro* folate deficiency induces deoxynucleotide pool imbalance, apoptosis and mutagenesis in Chinese hamster ovary cells. Cancer Res 1994;54:5075–80.
22. Bebenek K, Roberts JD, Kunkel TA. The effects of dNTP pool imbalances on frameshift fidelity during DNA replication. J Biol Chem 1992;267:3589–96.
23. Snyder RD. Effects of nucleotide pool imbalances on the excision repair of ultraviolet-induced damage in the DNA of human diploid fibroblasts. Basic Life Sci 1985;31:163–73.
24. Phear G, Meuth M. The genetic consequences of DNA precursor pool imbalance: sequence analysis of mutations induced by excess thymidine at the hamster aprt locus. Mutat Res 1989;214:201–6.
25. Zhang J, Cui Y, Kuang G, et al. Association of the thymidylate synthase polymorphisms with esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma. Carcinogenesis 2004;25:2479–85.

26. Graziano F, Kawakami K, Watanabe G, et al. Association of thymidylate synthase polymorphisms with gastric cancer susceptibility. Int J Cancer 2004;112:1010–4.

27. Zhang Z, Xu Y, Zhou J, et al. Polymorphisms of thymidylate synthase in the 5′- and 3′-untranslated regions associated with risk of gastric cancer in South China: a case-control analysis. Carcinogenesis 2005; 26:1764–9.

28. Zhang Z, Shi Q, Sturgis EM, Spitz MR, Hong WK, Wei Q. Thymidylate synthase 5′- and 3′-untranslated region polymorphisms associated with risk and progression of squamous cell carcinoma of the head and neck. Clin Cancer Res 2004;10:7903–10.

29. Shi Q, Zhang Z, Neumann AS, Li G, Spitz MR, Wei Q. Case-control analysis of thymidylate synthase polymorphisms and risk of lung cancer. Carcinogenesis 2005;26:649–56.

30. Yuan JM, Lu SC, Van Den Berg D, et al. Genetic polymorphisms in the methylenetetrahydrofolate reductase and thymidylate synthase genes and risk of hepatocellular carcinoma. Hepatology 2007;46: 749–58.

31. Kim SR, Ozawa S, Saito Y, et al. Fourteen novel genetic variations and haplotype structures of the TYMS gene encoding human thymidylate synthase (TS). Drug Metab Pharmacokinet 2006;21: 509–16.

32. Dolnick BJ. Cloning and characterization of a naturally occurring antisense RNA to human thymidylate synthase mRNA. Nucleic Acids Res 1993;21:1747–52.

33. Dolnick BJ, Black AR, Winkler PM, Schindler K, Hsueh CT. rTS gene expression is associated with altered cell sensitivity to thymidylate synthase inhibitors. Adv Enzyme Regul 1996;36:165–80.

34. Chu J, Dolnick BJ. Natural antisense (rTSα) RNA induces site-specific cleavage of thymidylate synthase mRNA. Biochim Biophys Acta 2002;1587:183–93.

35. Dolnick BJ, Angelino NJ, Dolnick R, Sufrin JR. A novel function for the rTS gene. Cancer Biol Ther 2003;2:364–9.

36. Xie W, Duan R, Chen I, Samudio I, Safe S. Transcriptional activation of thymidylate synthase by 17β-estradiol in MCF-7 human breast cancer cells. Endocrinology 2000;141:2439–49.