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

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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 GeneChip Gene and 5-kb flanking regions was done for 11 haplotype-tagging SNPs identified, 7 that are located 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 1,149 newly diagnosed endometrial cancer cases at ages 30 to 69 years
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, breastfeeding, or estrogen hormone use. Body mass index (weight [kg]/height [m\(^2\)]) and waist-to-hip circumference ratios 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 \(\geq 0.05\), 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 MegaAllele 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.

\(^4\) http://www.hapmap.org

\(\chi^2\) 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 the HaploView software (18), and haplotype blocks were defined using the methods of Gabriel et al. (19). Haplotype analyses were conducted using the 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; \(P_{\text{trend}} = 0.14\)). 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; \(P_{\text{trend}} = 0.03\)). However, the test for interaction was not significant (\(P_{\text{interaction}} = 0.24\); 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 rs9948553), 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).
| rs502396 | 0.280 | 1,027 | 1,003 | 1.0 | 256 (57.7) | 206 (53.4) | 1.0 | 299 (51.3) | 314 (30.9) | 1.0 | 234 (40.1) | 253 (33.9) | 1.0 (0.8-1.2) |
| CC | 555 (54.0) | 520 (51.8) | 1.0 | 156 (31.3) | 151 (39.1) | 0.8 (0.6-1.1) | 234 (40.1) | 253 (33.9) | 1.0 (0.8-1.2) |
| CT | 390 (38.0) | 406 (40.6) | 0.9 (0.7-1.1) | 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 |
| TT | 82 (8.0) | 77 (7.7) | 1.0 (0.7-1.4) | 1.0 (0.8-1.1) | 0.50 | 844 | 386 | 1.0 | 844 | 386 | 1.0 | 844 | 386 | 1.0 | 844 | 386 | 1.0 | 844 | 386 | 1.0 |
| rs2244500 | 0.310 | 1,028 | 1,003 | 1.0 | 237 (53.4) | 192 (49.7) | 1.0 | 272 (46.6) | 277 (44.9) | 1.0 | 247 (42.3) | 282 (45.7) | 0.9 (0.7-1.1) |
| CC | 509 (49.5) | 469 (46.8) | 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) |
| CT | 410 (39.9) | 444 (44.3) | 0.9 (0.7-1.0) | 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 |
| TT | 109 (10.6) | 90 (9.0) | 1.1 (0.8-1.5) | 1.0 (0.9-1.1) | 0.70 | 443 | 386 | 1.0 | 443 | 386 | 1.0 | 443 | 386 | 1.0 | 443 | 386 | 1.0 |
| rs3786362 | 0.188 | 683 (66.8) | 659 (65.9) | 1.0 | 278 (62.8) | 245 (63.5) | 1.0 | 405 (70.0) | 414 (67.4) | 1.0 | 154 (26.6) | 180 (29.3) | 0.9 (0.7-1.3) |
| TT | 305 (29.8) | 306 (30.6) | 1.0 (0.9-1.2) | 151 (34.1) | 126 (32.6) | 1.0 (0.9-1.2) | 154 (26.6) | 180 (29.3) | 0.9 (0.7-1.3) | 154 (26.6) | 180 (29.3) | 0.9 (0.7-1.3) | 154 (26.6) | 180 (29.3) | 0.9 (0.7-1.3) | 154 (26.6) | 180 (29.3) | 0.9 (0.7-1.3) |
| rs3786362 | 0.108 | 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 |
| rs3819102 | 0.287 | 499 (48.6) | 463 (46.2) | 1.0 | 231 (52.1) | 188 (48.7) | 1.0 | 268 (45.9) | 275 (44.6) | 1.0 | 250 (42.8) | 279 (45.2) | 0.9 (0.7-1.2) |
| CC | 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) |
| CT | 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 |
| 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 |

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.
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 the 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 rs999517, 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 (rTS\(\alpha\) and rTS\(\beta\)) 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 |
|-----------------|-----------------|-----------------|-----------------|
|                  | OR (95% CI)*     | OR (95% CI)†    |                  |
|                  | Dominant model   | Recessive model | Additive model   |
| TYMS block 1     |                 |                 |                 |
| TTCT             | 1.0 (0.8-1.3)    | 1.0 (0.8-1.2)   | 1.0 (reference) |
| CTCT             | 1.0 (0.8-1.2)    | 1.0 (0.7-1.4)   | 1.0 (0.8-1.1)   |
| TACC             | 0.9 (0.8-1.1)    | 1.2 (0.8-1.8)   | 0.9 (0.8-1.1)   |
| TCTC             | 1.0 (0.8-1.2)    | 1.1 (0.7-1.7)   | 1.0 (0.8-1.2)   |
| Others           | 1.4 (0.9-2.1)    |                 |                 |
| TYMS block 2     |                 |                 |                 |
| TCG              | 1.0 (0.8-1.2)    | 1.1 (0.9-1.3)   | 1.0 (reference) |
| TTC              | 1.0 (0.8-1.2)    | 0.9 (0.8-1.3)   | 0.9 (0.8-1.1)   |
| ATC              | 0.9 (0.8-1.1)    | 1.2 (0.8-1.7)   | 1.0 (0.8-1.2)   |
| TTG              | 0.8 (0.6-1.0)    | 1.0 (0.4-2.3)   | 0.8 (0.7-1.1)   |
| Others           | 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.

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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.

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