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The association between two polymorphisms in the TYMS gene and breast cancer risk: a meta-analysis

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Abstract Thymidylate synthase (TYMS), which catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate, is a central enzyme in the folate metabolic pathway. Epidemiological studies have evaluated the association between TYMS gene polymorphisms and breast cancer susceptibility; however, the published data are still inconclusive. To derive a more precise assessment of this relationship, we performed a meta-analysis based on currently available data by searching PubMed, EMBASE databases, and the Cochrane Library. A total of 10 eligible studies were identified for the TYMS TSER polymorphism (six studies with 2,718 cases and 3,423 controls) and for the TYMS TS3′-UTR polymorphism (five studies with 1,969 cases and 2,290 controls). The overall odds ratio (OR) and the corresponding 95% confidence interval (CI) showed a statistical association between the TSER polymorphism and breast cancer risk under homozygote comparison (2R/2R vs. non-2R/non-2R; OR 1.25; 95% CI 1.04–1.50), allele contrast (2R vs. non-2R; OR 1.09; 95% CI 1.01–1.19) and the recessive model (OR 1.19; 95% CI 1.01–1.39). In the subgroup analysis by ethnicity, a statistically significant increase in cancer risk was found among Caucasians for homozygote comparison (OR 1.31; 95% CI 1.10–1.57), the allele contrast model (OR 1.12; 95% CI 1.02–1.23) and the dominant model (OR 1.40; 95% CI 1.00–1.95). For the TS3′-UTR polymorphism, significant effects were shown using the allele contrast model (OR 1.33; 95% CI 1.03–1.73). However, the TS3′-UTR polymorphism increased breast cancer risk among Asian women (del6 vs. ins6; OR 1.41; 95% CI 1.01–1.98) but not Caucasian women using the homozygote comparison. In conclusion, our meta-analysis suggests that the TSER polymorphism may increase susceptibility to breast cancer in the Caucasian population and the TS3′-UTR polymorphism may be a genetic determinant for developing breast cancer in the Asian population; therefore, ethnic background should be carefully considered in further studies.

Keywords Breast cancer · Polymorphism · Thymidylate synthase · Meta-analysis

Introduction

Breast cancer is the second leading cause of death by cancer in women and is the most common cancer among women across the world [1]. Some epidemiological studies indicate that various types of genetic damage induced by endogenous metabolites and exogenous hazards may play an important role in the development of breast cancer [2]. 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, DNA synthesis, and DNA repair [3]. Several large, prospective epidemiological studies have implicated folate deficiency in the development of several cancers [4–8].

Many key enzymatic regulators are involved in folate metabolism. Among them, thymidylate synthase (TYMS) catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in DNA synthesis, using 5, 10-methylenetetrahydrofolate as a methyl donor. This process is essential for the synthesis of thymidine, a nucleotide needed for DNA synthesis and

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repair [9]. TYMS is also the target for the widely used chemotherapeutic agent 5-fluorouracil (5-FU) [10]. Recently, it has been shown that functional polymorphisms in the TYMS gene may contribute to breast cancer risk via effects on nucleotide synthesis. This is particularly true if the genetic variation is combined with low levels of folate or coenzymes of folate metabolism (vitamins B12 and B6) [8]. Therefore, genetic alterations in TYMS enzyme efficiency and/or expression level may result in individual susceptibility to breast and other cancers.

A tandem-repeat polymorphism has been identified in the TYMS promoter enhancer region (TSER), which contains triple (3R) or double (2R) repeats of a 28-bp sequence and several rare alleles containing 4, 5, or 9 repeats [11]. In vitro and in vivo studies show that TYMS expression is TSER genotype-dependent and that the 3R allele is associated with an increase in TYMS expression [12]. Recently, a novel, potentially functional polymorphism within the 3′-UTR of the TYMS gene has also been identified, which consists of a 6-bp deletion/insertion (del6/ins6) at nucleotide 1494 of the TYMS mRNA (TS3′-UTR). This polymorphism may affect RNA stability and reduce TYMS protein expression [13].

Considering the potential influence of altering TYMS activation on folate metabolism, many epidemiological studies have explored the association between these two TYMS variants and increased breast cancer risk development [14–23]. However, the results remain inconclusive, which may be due to limitations in individual studies. Therefore, we performed a meta-analysis of 10 published case–control studies covering 4,443 cases and 5,447 controls to obtain a more precise estimation of the relationship between TYMS polymorphisms and breast cancer risk.

Methods

Search strategy

We searched the literature from PubMed, EMBASE, and the Cochrane Library to identify relevant and available published articles. The keywords and subject terms "thymidylate synthase," "TYMS," "TS," "polymorphism," and "breast cancer'' and combinations of these phrases were used for the search (last search: July 31, 2010). The language that the papers were written in was not restricted. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Review articles and bibliographies of other identified, relevant studies were searched by hand for additional eligible studies. Only published studies with full text articles were included. The following criteria were used to select the eligible studies: (a) a case–control study on the association between TYMS polymorphisms and breast cancer risk, (b) an available genotype or allele frequency, (c) number of different genotypes for estimating an odds ratio (OR) with a 95% confidence interval (CI), and (d) a genotype distribution among the control populations consistent with Hardy–Weinberg Equilibrium (HWE). When authors reported two or more publications on possibly the same patient populations, only the most recent or complete study was included in the review to avoid overlap between the cohorts.

Data extraction

Two reviewers (J. Wang and J. Bi) independently evaluated the final articles selected for further meta-analysis. Disagreements were resolved by discussions between the two authors. If they could not reach a consensus, a third investigator (B. Wang) resolved the disagreements. Reviews, non-original articles, non-case–control studies, and studies involving breast cancer cells and animal models were excluded from this meta-analysis. Data retrieved from the reports included the following: first author's name, publication year, country of origin, source of controls, racial decent of the study population (categorized as Caucasian and Asian descents), genotyping method, eligible and genotyped cases and controls, the number of cases and controls for each TYMS single-nucleotide polymorphism (SNP) genotype, and the minor allele frequency for the controls. In each study, we did not define a minimum number of patients for inclusion in this meta-analysis.

Statistical methods

Crude ORs and their corresponding 95% CIs were used to assess the strength of association between the studied TSER polymorphism and breast cancer susceptibility. Five different ORs were calculated using the following models: the dominant genetic model (2R/2R + non-2R/2R vs. non-2R/non-2R), the recessive genetic model (2R/2R vs. non-2R/non-2R + non-2R/2R), the homozygote comparison (2R/2R vs. non-2R/non-2R), the allele contrast model (2R vs. non-2R), and the non-2R/2R vs. non-2R/non-2R comparison. The same methods were applied to the analysis of the TS3′-UTR polymorphism.

The χ^2-based Q statistic was used to investigate the degree of heterogeneity between the studies, and a P value <0.1 was interpreted as significant heterogeneity among the studies [24]. The I^2 index expresses the percentage of the total variation across studies due to heterogeneity. I^2 values of 25, 50, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively. When there was no statistical heterogeneity, we used a fixed-effects

model (Mantel–Haenszel method). If heterogeneity was present, we used a random-effects model (DerSimonian–Laird method) to account for inter-study heterogeneity. HWE was tested by the χ^2 test. All statistical analyses were performed using Review Manage (Version 5.0; Oxford, England).

Results

Study characteristics

A total of 10 studies (4,443 cases and 5,447 controls) met the inclusion criteria (Table 1). In these studies, a total of four TYMS polymorphisms were reported, and the most studied genetic variants were TSER and TS3′-UTR; however, one study did report the association between rs16948322 or rs2298581 and breast cancer risk [20]. Therefore, our meta-analysis focused on the two genetic variants TSER and TS3′-UTR. A total of nine studies were included in the meta-analysis for the TSER (2,718 cases and 3,423 controls) [15, 16, 18, 21–23] and TS3′-UTR polymorphisms (1,969 cases and 2,290 controls) [14, 17–19, 23]. These studies were published between 2004 and 2010. All studies were carried out in various ethnic populations, including four studies in Asian populations [17, 20, 21, 23] and six studies in Caucasian populations [14–16, 18, 19, 22]. The main characteristics of these studies are summarized in Tables 2 and 3 for the TSER and TS3′-UTR polymorphisms, respectively. Several genotyping methods were used, and they included the polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP), the TaqMan assay, and the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Except for two studies, the genotype distributions in the study controls were in agreement with HWE [14, 18].

Meta-analysis

The results of this meta-analysis are shown in Table 4. As there was no statistical heterogeneity for the TSER polymorphism ($P = 0.67$, $I^2 = 0$), we used a fixed-effects model. The 2R allele was significantly associated with breast cancer risk compared to the non-2R allele (OR 1.09; 95% CI 1.01–1.19). Breast cancer risks were also significantly increased using the homozygote comparison (OR 1.25; 95% CI 1.04–1.50) and the recessive model (OR 1.19; 95% CI 1.01–1.39). No significant association was found between the TSER polymorphism and breast cancer risk in the overall population using the dominant model (OR 1.05; 95% CI 0.92–1.19) or the 2R/non-2R vs. non-2R/non-2R comparison (OR 1.23; 95% CI 0.91–1.65). Whether genotype frequencies in the control population were consistent with HWE or not, when all subjects were included, we also found significant associations between the TSER polymorphism and breast cancer risk under allele contrast (OR 1.13; 95% CI 1.05–1.23) and homozygote comparison models (OR 1.30; 95% CI 1.10–1.53).

In the subgroup analysis by ethnicity, the differences between the allele, homozygote, recessive, and dominant models were insignificant in the Asian population. However, significantly increased risks were observed among Caucasian women (allele contrast: OR 1.13; 95% CI 1.02–1.25; 2R/2R vs. non-2R/non-2R comparison: OR 1.26; 95% CI 1.03–1.53; and dominant model: OR 1.17; 95% CI 1.00–1.36).

For the TS3′-UTR polymorphism, the association between this genetic variant and breast cancer risk was significant under homozygote comparison (OR 1.33; 95% CI 1.03–1.73). We found no significant associations between this genetic variant and breast cancer risk in the other comparison models tested (allele contrast: OR 1.07; 95% CI 0.96–1.19; for ins6/del6 vs. ins6/ins6: OR 1.26; 95% CI 0.85–1.86; recessive model del6/del6 vs. (ins6/ins6 + ins6/del6): OR 0.93; 95% CI 0.71–1.22; and

Table 1 Characteristics of all eligible studies considered in the meta-analysis

First author	Year	Country	Ethnicity	Study design	Genotyping method	Cases	Control	Studied SNP
Sangrajrang [20]	2010	Thailand	Asian	Hospital	TaqMan assay	507	497	rs16948322, rs2298581
Jakubowska [18]	2010	Poland	Caucasian	Hospital	PCR-RFLP	319	290	TSER, TS3′-UTR
Henríquez-Hernández [16]	2009	Spain	Caucasian	Hospital	PCR-RFLP	135	304	TSER
Inoue [17]	2008	Singapore	Asian	Population	TaqMan assay	380	662	TS3′-UTR
Sukuki [21]	2008	Japan	Asian	Hospital	TaqMan assay	456	912	TSER
Xu [22]	2007	USA	Caucasian	Population	TaqMan assay	1053	1099	TSER
Akisik [14]	2007	Turkey	Caucasian	Population	PCR-RFLP	150	141	TS3′-UTR
Zhai [23]	2006	China	Asian	Hospital	TaqMan assay	432	473	TSER, TS3′-UTR
Justenhoven [19]	2005	Germany	Caucasian	Population	MALDI-TOF MS	688	724	TS3′-UTR
Grieu [15]	2004	Australia	Caucasian	Population	TaqMan assay	323	345	TSER

Table 2 Distribution of TSER genotype among breast cancers of cases and controls included in the meta-analysis

First author	Year	Ethnicity	Genotype							Allele			
			Case			Control				Case		Control	
			2R/2R	2R/3R	3R/3R	2R/2R	2R/3R	3R/3R	HWE (*P*)	2R	3R	2R	3R
Jakubowska [18]	2010	Caucasian	66	158	90	63	87	140	0.00	290	338	213	367
Henríquez-Hernández [16]	2009	Caucasian	44	59	30	66	145	72	0.67	147	119	277	289
Sukuki [21]	2008	Asian	16	109	329	20	239	651	0.72	141	767	279	1541
Xu [22]	2007	Caucasian	230	512	311	211	532	356	0.63	972	1134	954	1244
Zhai [23]	2006	Asian	23	130	279	25	143	305	0.13	176	688	193	753
Grieu [15]	2004	Caucasian	70	167	86	76	161	108	0.28	307	339	313	377

Table 3 Distribution of TS3′-UTR genotype among breast cancers of cases and controls included in the meta-analysis

First author	Year	Ethnicity	Genotype							Allele			
			Case			Control				Case		Control	
			ins6/ins6	ins6/del6	del/del	ins6/ins6	ins6/del6	del6/del6	HWE (*P*)	del6	ins6	del6	Ins6
Jakubowska	2010	Caucasian	166	144[a]		134	156[a]						
Inoue	2008	Asian	30	157	193	61	273	328	0.70	543	217	929	395
Akisik	2007	Caucasian	53	77	20	29	84	28	0.02	117	183	140	142
Zhai	2006	Asian	30	208	194	56	201	216	0.38	596	268	633	313
Justenhoven	2005	Caucasian	272	245	58	292	267	51	0.36	361	789	369	851

[a] ins6/del6 + del6/del6

dominant model del6/del6 + ins6/del6 vs. ins6/ins6: OR 1.03; 95% CI 0.80–1.33). However, when stratified by ethnicity, a significantly elevated risk among Asian women was found using the homozygote comparison (OR 1.41; 95% CI 1.01–1.98). When studies with genotype frequencies in the control population were not consistent with HWE and were also included [14], the results were in agreement with the findings from the foregoing analysis.

Discussion

In the present study, we collected all available, published studies and performed a meta-analysis to examine the association between the TYMS polymorphisms TSER and TS3′-UTR and susceptibility to breast cancer. We performed this study to clarify controversial results from previous reports. Six and five studies on the TSER and TS3′-UTR genetic genotypes, respectively, were critically reviewed. Our meta-analysis showed that breast cancer risk was significantly increased in TSER 2R genotype carriers. Our data also indicated that the TS3′-UTR del6/del6 genotype might be a risk factor for breast cancer susceptibility in Asian individuals, but it did not show a significant role in all subjects or in the Caucasian population.

Folate has a central role in one-carbon metabolism and is a critical coenzyme for both nucleotide synthesis and methylation reactions [3]. Folate depletion alone is sufficient to diminish the methyl pool, and a high level of dietary folate intake is associated with a decreased risk for several common cancers, including breast cancer [4–8]. Enzymes such as methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTH), and TYMS play key roles in one-carbon and folate metabolisms. Previously, functional polymorphisms in genes of these folate-related enzymes were found to contribute to the alteration in folate metabolism and were independently associated with breast cancer risk [5, 20, 21, 25, 26]. TYMS is a key enzyme in the nucleotide biosynthetic pathway that converts the methylation of dUMP to dTMP using 5, 10-methylene tetrahydrofolate as a cofactor. This reaction is a key source of de novo cellular thymidylate production [9]. A SNP in the 5′ tandem repeats of the TYMS gene (TSER) has been discovered. Individuals with the wild-type form of 3R show higher transcription of TYMS than those with the variant form that includes 2R and 4, 5, or 9 repeats [27]. In addition, another novel polymorphism of TS3′-UTR, consisting of a 6-bp deletion at nucleotide 1494 of the TYMS mRNA, has been associated with decreased mRNA stability, an enhanced rate of mRNA decay and lower tumor TYMS

Table 4 Summary of ORs with 95% CIs for ordinary genetic contrasts of the association of the TYMS polymorphisms, TSER and TS3′-UTR and breast cancer risk

Contrast	Overall or subgroup	Fixed effects OR (95% CI)	Radom effects OR (95% CI)	I^2 for heterogeneity (%)	*P* value for heterogeneity	*P* value for *Z* test
TSER in breast cancer						
2R/2R vs. non-2R/non-2R	All	1.30 (1.10–1.53)	1.30 (1.10–1.53)	0	0.74	0.002
	All in HWE	1.25 (1.04–1.50)	1.25 (1.04–1.50)	0	0.82	0.01
	Asian	1.22 (0.78–1.90)	1.22 (0.78–1.90)	0	0.32	0.38
	Caucasian	1.26 (1.03–1.53)	1.31 (1.10–1.57)	0	0.77	0.02
2R/non-2R vs. non-2R/non-2R	All	1.16 (1.03–1.30)	1.23 (0.91–1.65)	82	<0.001	0.18
	All in HWE	1.05 (0.92–1.19)	1.05 (0.92–1.19)	0	0.54	0.47
	Asian	0.94 (0.78–1.14)	0.94 (0.78–1.14)	0	0.65	0.55
	Caucasian	1.13 (0.96–1.33)	1.13 (0.96–1.33)	0	0.61	0.15
2R/2R vs. non-2R/non-2R + non-2R/2R	All	1.15 (1.00–1.33)	1.15 (1.00–1.33)	1	0.41	0.06
	All in HWE	1.19 (1.01–1.39)	1.19 (1.01–1.39)	1	0.40	0.03
	Asian	1.23 (0.79–1.91)	1.23 (0.79–1.91)	11	0.29	0.35
	Caucasian	1.18 (1.00–1.40)	1.19 (0.95–1.49)	31	0.23	0.12
2R/2R + non-2R/2R vs. non-2R/non-2R	All	1.18 (1.06–1.32)	1.23 (0.97–1.56)	75	0.001	0.09
	All in HWE	1.09 (0.96–1.22)	1.09 (0.96–1.22)	0	0.64	0.17
	Asian	0.97 (0.81–1.17)	0.97 (0.81–1.17)	0	0.83	0.77
	Caucasian	1.17 (1.00–1.36)	1.17 (1.00–1.36)	0	0.89	0.05
2R vs. non-2R	All	1.13 (1.05–1.23)	1.11 (1.01–1.21)	39	0.15	0.002
	All in HWE	1.09 (1.01–1.19)	1.09 (1.01–1.19)	0	0.67	0.03
	Asian	1.01 (0.86–1.18)	1.01 (0.86–1.18)	0	0.92	0.93
	Caucasian	1.13 (1.02–1.25)	1.13 (1.02–1.25)	0	0.63	0.02
TS3′-UTR in breast cancer						
del6/del6 vs. ins6/ins6	All	1.16 (0.91–1.24)	1.06 (0.65–1.71)	72	0.01	0.88
	All in HWE	1.33 (1.03–1.73)	1.33 (1.03–1.73)	0	0.54	0.03
	Asian	1.41 (1.01–1.98)	1.41 (1.01–1.98)	0	0.33	0.05
	Caucasian	0.93 (0.65–1.32)	0.72 (0.24–2.19)	86	0.008	0.56
ins6/del6 vs. ins6/ins6	All	1.03 (0.863–1.24)	1.04 (0.66–1.62)	78	0.004	0.87
	All in HWE	1.14 (0.94–0.38)	1.26 (0.85–1.86)	67	0.05	0.26
	Asian	1.50 (1.07–2.11)	1.50 (0.92–2.45)	52	0.15	0.11
	Caucasian	0.88 (0.71–1.10)	0.74 (0.38–1.24)	80	0.03	0.36
del6/del6 vs. ins6/ins6 + ins6/del6	All	0.88 (0.75–1.04)	0.89 (0.69–1.14)	52	0.10	0.13
	All in HWE	0.90 (0.77–1.07)	0.93 (0.71–1.22)	60	0.08	0.61
	Asian	0.85 (0.70–1.02)	0.85 (0.65–1.11)	53	0.14	0.22
	Caucasian	1.01 (0.73-1.14)	0.91 (0.47–1.78)	69	0.01	0.79
del6/del6 + ins6/del6 vs. ins6/ins6	All	0.94 (0.81–1.10)	0.92 (0.68–1.26)	70	0.009	0.61
	All in HWE	1.00 (0.85–1.18)	1.03 (0.80–1.33)	53	0.10	0.82
	Asian	1.32 (0.94–1.83)	1.32 (0.94–1.83)	0	0.51	0.10
	Caucasian	0.85 (0.72–1.02)	0.76 (0.51–1.11)	75	0.02	0.16
del6 vs. ins6	All	1.02 (0.92–1.13)	0.99 (0.83–1.18)	63	0.04	0.45
	All in HWE	1.07 (0.96–1.19)	1.07 (0.96–1.19)	0	0.95	0.22
	Asian	1.08 (0.94–1.24)	1.08 (0.94–1.24)	0	0.82	0.27
	Caucasian	0.95 (0.81–1.11)	0.84 (0.53–1.36)	85	0.01	0.49

expression. Thus, functional genetic variants of the TYMS gene may represent risk factors for breast cancer because of their central role in cellular folate metabolism.

Previous studies showed that the TS3′-UTR variant is associated with several types of cancer risk [28, 29]; however, the results remain mixed. In contrast, the TSER

genotype appears to be a protective factor against bladder cancer development [30]. Only Henríquez-Hernández et al. reported a significant and increased risk for breast cancer in women who were homozygous for TSER 2R (OR 1.63; 95% CI 1.05–2.52) [16]. In a Chinese population, Zhai et al. found that a significantly reduced risk for breast cancer was associated with the ins6/ins6 homozygous variant (OR 0.58; 95% CI 0.35–0.97) [23]. Our meta-analysis confirmed a significant association between the TSER polymorphism and breast cancer risk in Caucasian women. However, based on currently available data, there is a lack of evidence supporting that the TSER variant genotype is associated with an individual's susceptibility to breast cancer in the Asian population. In addition, this meta-analysis revealed a modest association between the TS3′-UTR polymorphism and breast cancer risk in Asian women. Thus, the same polymorphisms may play varying roles in different ethnicities due to differences in genetic backgrounds and environmental exposure. Sangrajrang et al. examined the TYMS rs16948322 and rs2298581 polymorphisms and found that they were not risk factors for developing breast cancer (rs16948322: OR 1.14, 95% CI 0.87–1.50; rs2298581: OR 0.95, 95% CI 0.72–1.26) [20]. Although results of the study conducted by Xu et al. found that the rs2298581 SNP was associated with a marginally significant decrease in endometrial cancer risk [31], our collected data were not sufficient to determine the effects of the two TYMS SNPs on breast cancer risk because few studies exist on these SNPs together. Therefore, the roles of rs16948322 and rs2298581 polymorphisms in breast cancer risk need further investigation.

Presumably, genetic factors alone have weak effects on an individual's phenotype. These effects may be measurable, except in the context of some environmental factors, such as smoking, aging, and alcohol, green tea, and folate intake. Inoue et al. suggested that the MTHFR/TYMS genotypes influence breast cancer risk in women, and their effects may be modified by weekly/daily green tea intake [17]. In bladder cancer, Rouissi et al. reported the correlation between TYMS gene variations and bladder cancer risk among smokers [30]. The lung cancer risk associated with the TS3′-UTR genotype was more pronounced in older people, current and heavy smokers and current drinkers [28]. However, green tea intake, tobacco smoking and drinking have different effects on different cancer types. No association study has been restricted to populations exposed to only one environmental factor; therefore, it is difficult to examine the interaction between the TYMS polymorphisms and dietary and environment factors in breast cancer development.

The interaction of different polymorphisms in the same gene or between different genes might contribute to breast cancer risk. The combined effects of TYMS polymorphisms and other genetic polymorphisms, including those in MTHFR and MTR, which are involved in folate metabolism or one-carbon metabolism, were evaluated in breast cancer [16, 17] and other types of cancer risk [30]. A case–control analysis in a Chinese population revealed no statistical evidence for interactions between the combined variant genotypes in the TYMS gene and risks for breast cancer [23]. We evaluated the combined effects of these polymorphisms on susceptibility to breast cancer, and unfortunately, the data were not sufficient for simply putting them together in a meta-analysis.

Heterogeneity for the TSER and TS3′-UTR polymorphisms was observed among these studies. The heterogeneity may be due to various factors, such as diversity in the population characteristics, differences in the number of cases and controls, genotyping methods and study design. To eliminate heterogeneity, we placed the studies in subgroups. When significant heterogeneity was present, we used a random-effects model to pool the results. There was one study each for TSER [18] and TS3′-UTR [14] that was derived from the HWE, which may contribute to selection bias. In addition, some unpublished, eligible publications were not available in the present meta-analysis, which could have affected the results.

In conclusion, we found several significant associations between the TYMS polymorphisms and breast cancer risk. For the TSER polymorphism, risk effects were found under some genetic models. In addition, the TSER polymorphism might increase breast cancer risk in Caucasian women, which indicates a difference between populations. We also found the TS3′-UTR del6/del6 genotype might be a risk factor in the susceptibility of breast cancer in Asian individuals under the homozygote comparison. However, studies based on larger, stratified case–control populations are still needed to clarify the different effects of the two key TYMS polymorphisms in Asian and Caucasian women. Also, studies examining the combined effects of the TSER and TS3′-UTR polymorphisms or different polymorphisms in enzymes involved in folate metabolism should be investigated.

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