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Genetic Variant rs16430 6bp > 0bp at the microRNA-Binding Site in *TYMS* and Risk of Sporadic Breast Cancer Risk in Non-Hispanic White Women Aged 55 Years

Xiaoxiang Guan^{1,2}, Hongliang Liu³, Jingfang Ju⁴, Yangkai Li³, Peng Li⁵, Li-E Wang¹, Abenaa M. Brewster⁶, Thomas A. Buchholz⁵, Banu K. Arun⁷, Qingyi Wei^{3,*}, and Zhensheng Liu^{3,*}

¹Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

²Department of Medical Oncology, Jinling Hospital, Nanjing University School of Medicine, Jiangsu, China

³Duke Cancer Institute, Duke University Medical Center, Durham, North Carolina

⁴Department of Pathology, Stony Brook University, Stony Brook, New York

⁵Department of Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

⁶Department of Clinical Cancer Prevention, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

⁷Department of Breast Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Thymidylate synthase (*TYMS*) is involved in the folate metabolism and provision of nucleotides needed for DNA synthesis and repair. Thus, functional genetic variants in *TYMS* may alter cancer risk. In the study, we evaluated associations of three germline variants (rs2790 A > G, rs16430 6 bp > 0 bp, and rs1059394 C > T) in the predicted miRNA-binding sites of *TYMS* with risk of sporadic breast cancer in non-Hispanic white women aged 55. We found that carriers of the rs16430 0 bp variant allele had an increased risk of breast cancer [adjusted odd ratio (OR) = 1.37, 95% confidence interval (CI): 1.08–1.73; $P = 0.010$], compared with carriers of the 6 bp/6 bp genotype. This increased risk was more evident in older subjects (OR = 1.47, 95% CI = 1.06–2.03, $P = 0.022$), never smokers (OR 1.67, 95% CI = 1.23–2.25, $P < 0.001$), never drinkers (OR = 1.44, 95% CI 1.01–2.05, $P = 0.043$), and estrogen receptor-positive patients (OR = 1.46, 95% CI = 1.11–1.92, $P = 0.006$), regardless of tumor stages. The results are consistent with the functional analyses of rs16430 as previously reported, which showed that the 0bp allele had a decrease in both

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*Correspondence to: Duke Cancer Institute, Duke University Medical Center, Durham, NC 27710. Xiaoxiang Guan and Hongliang Liu contributed equally to this work.

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luciferase activity by ~70% and mRNA levels by ~50% compared with the 6bp allele. Additionally, the rs16430 variant was predicted to influence the binding activity of *miR-561*. Taken together, these findings indicate that the *TYMS* rs16430 may contribute to the etiology of sporadic breast cancer in non-Hispanic white women aged 55 yr. Further validation in large population-based or cohort studies is needed.

Keywords

genetic susceptibility; molecular epidemiology; microRNA; *miR-561*; *TYMS*; breast cancer

INTRODUCTION

Breast cancer is the most common cancer among women in the United States, and approximately 232,340 new cases will be diagnosed, resulting in 39,620 deaths in 2013 [1]. It is well known that the etiology of breast cancer is a complex one, with a combination of genetic, environmental, hormonal and reproductive factors, such as earlier menarche, nulliparity, later age at first birth, and later menopause [2–4]. Recently, increasing evidence has shown that genetic susceptibility also contributes to the development of disease [5–7]. For example, it has been reported that there is an etiologic association between germline variants in thymidylate synthase (*TYMS*) and risk of breast cancer [8,9].

TYMS is a key enzyme that catalyzes the conversion of deoxyuridine-5'-monophosphate (dUMP) to deoxythymidine-5'-monophosphate (dTTP), which is the essential precursor of DNA synthesis and repair process [10,11]. Impairment of the *TYMS* enzyme is associated with increased chromosome damage and fragile site induction [12,13], suggesting that DNA repair mechanisms can be affected by nucleotide availability associated with *TYMS* down regulation or a poorer response to DNA repair, which may lead to transformation and cancer development. Therefore, *TYMS* plays an important role in tumorigenesis, cell senescence, and apoptosis [14,15] that are known to be associated with cancer risk, including breast cancer [16–19]. In addition, *TYMS* is also an essential enzyme in proliferating cells that require high levels of folate, and it is an important target for a variety of fluoropyrimidine-based chemotherapeutic drugs, including 5-FU that is used to treat breast cancer [10,20]. Resistance to fluoropyrimidines can occur through different mechanisms, including elevated *TYMS* protein expression as a result of increased transcription [21] and translation rates [22]. Clinical studies have also demonstrated that the *TYMS* expression levels of a tumor predict the response to 5-FU-based chemotherapy, in which a high *TYMS* expression provides the resistance to 5-FU and its derivatives [23,24].

Recent studies have indicated that microRNAs (miRNAs) also play an important role in cancer development. miRNAs are single-stranded 21–23 nucleotide long RNA molecules that can negatively regulate target gene translation through binding to partially complementary sequences in the 3' untranslated region (UTR) of their target messenger RNAs (mRNAs) [25,26]. This process results in either the degradation of target mRNAs or repression of their translation. Elevated or decreased expression of miRNAs has been found in tumors of various types, and miRNAs may function as tumor suppressor genes and/or

oncogenes in many cancers, including breast cancer [27–30]. It is reported that miRNAs play important roles in breast cancer progression by downregulating the expression of their target genes [29]. Recently, for example, Kheiriseid et al. [31] identified that *miR-561* can be a predictor of response to neoadjuvant chemoradiation therapy in colorectal cancer. More recently, Bastos et al. [32] found altered expression of *miR-561* in tumors of early onset breast cancer tumors patient. Furthermore, germline variants located in the predicted miRNA-binding sites (i. e., miRNA-binding SNPs) of the target genes may affect the regulation and function of miRNA-mediated genes and are, thus, associated with cancer susceptibility [33].

TYMS has been mapped to chromosome 18p11.32, and approximately 483 SNPs or variants in this gene are reported in the dbSNPs build 37.1 database (last search date: July 18, 2012; dbSNP database; http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?choosers=all&go=Go&locusId=836). Based on the TargetScan online tool [34], there are at least 19 SNPs or variants in the 3' UTR of *TYMS*, of which 13 are located in the predicted miRNAs-binding sites (<http://snpinfo.niehs.nih.gov/snpfunc.htm>). However, only three of these 13 variants [rs2790 G allele (*miR-1248*), rs16430 0 bp allele (*miR-561*) and rs1059394 T allele (*miR-892a*)] are reported to be common (i.e., minor allele frequency >0.05) in Caucasians (Fig. 1). Particularly, the rs16430 0bp/6bp variant, located in the 3'UTR of the *TYMS* primary transcript, is thought to influence *TYMS* mRNA stability and tumor mRNA level [20,35]. In the present study, we tested the hypothesis that germline variants in the predicted miRNA-binding sites of *TYMS* may modulate translation of *TYMS* mRNA and may, thus, be associated with risk of breast cancer. Therefore, we genotyped three common SNPs/variants reported to be in the predicted miRNA binding sites in non-Hispanic white women 55 yr, a unique study population in which we previously evaluated the role of other genetic variations in risk of developing breast cancer [5,36].

MATERIALS AND METHODS

Study Populations

As described previously [5], all subjects in this study were non-Hispanic white women. The 543 patients were diagnosed with primary breast cancer and recruited at The University of Texas M.D. Anderson Cancer Center between 1998 and 2006. These cases were histopathologically confirmed and untreated before the blood drawing. Patients with a previous cancer history or multiple primary neoplasms at the time of diagnosis were excluded from this study. During the same time period, we recruited 580 self-reported cancer-free controls from hospital visitors (i. e., friends, spouses or relatives) who were genetically unrelated with the cases of this study. Controls were frequency-matched to the cases by age (± 5 yr). Participants who had smoked at least 100 cigarettes in their lifetime were assigned as ever smokers and the remaining as never smokers. Participants who had consumed alcoholic beverage at least once a week for 1 yr in their lifetime were assigned as ever drinkers and the remaining as never drinkers. Additional information about expression status of estrogen receptor (ER)/progesterone receptor (PR), menopausal status, age at first full-term pregnancy, number of live births, use of hormone replacement therapy or oral contraceptives, and body mass index was available in the clinical database only for

the cases. For all subjects, a 30-ml of blood sample was collected after the informed consent was signed by each participant. The participation rate of those subjects who were approached for participation was about 90% for the cases and about 70% for the controls. The study protocol was approved by The University of Texas M. D. Anderson Cancer Center institutional review board.

Genotyping of *TYMS*

We extracted genomic DNA from the buffy coat fraction by centrifugation of 1 ml of whole blood, using a blood DNA mini kit (Qiagen, Inc.) according to the manufacturer's instructions. The DNA purity and concentration were determined by spectrophotometer measurement of absorbance at 260 and 280 nm. Genotyping of *TYMS* (rs2790 A > G and rs1059394 C > T) were performed using the TaqMan methodology in 384-well plates and read with the Sequence Detection Software on an ABI-Prism 7900 instrument according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Primers and probes were also supplied by Applied Biosystems. Each plate included four negative controls (no DNA), duplicated positive controls, and eight repeat samples. Amplification was done under the following conditions: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. Because the TaqMan assay could not be designed for the rs16430 6bp > 0bp variant, we genotyped it by using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method as previously described [16]. For all genotypes of the *TYMS* gene, the assay success rate was >99%, and the concordance rate for repeated assays of a 10% sample was 100%.

Statistical Analysis

The distribution of *TYMS* genotypes and selected risk factors between cases and controls were compared by using chi-square test. Hardy–Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$, where p is the frequency of the variant allele and $q = 1 - p$) was tested by a goodness-of-fit chi-square test in cancer-free controls. The association between *TYMS* genotypes and breast cancer risk was evaluated by calculating the odds ratio (ORs) and 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses. We also used the false-positive report probability (FPRP) to test for false positive associations [37]. For all significant genetic effects observed in our study, we calculated FPRP with prior probabilities of 0.0001, 0.001, 0.01, 0.1, and 0.25. The OR was set close to the observed value in our study, and a probability <0.2 was considered as noteworthy. All tests were two sided and $P < 0.05$ was considered significant. All statistical analyses were performed with SAS software (version 9.1.3; SAS Institute, Inc., Cary, NC), unless stated otherwise.

RESULTS

Characteristics of the Study Population

This study included 543 breast cancer cases and 580 cancer-free controls, because we used frequency matching by age, there was no significant difference in the distribution of age ($P = 0.995$). The cases were more likely to be smokers ($P = 0.030$) and drinkers ($P = 0.096$) than the controls (Table 1). These variables were also used in multivariate logistic regression

models to further adjust for any possible residual confounding effect on the association between selected *TYMS* SNPs and risk of breast cancer.

Distribution of *TYMS* Genotypes and Risk of Breast Cancer

The genotype distributions of *TYMS* rs2790 A > G, rs16430 6bp > 0bp and rs1059394 C > T are presented in Table 2. The observed genotype frequencies of these SNPs/variants were all in agreement with the Hardy-Weinberg equilibrium in the controls ($P = 0.779$ for rs2790 A > G, $P = 0.068$ for rs16430 6bp > 0bp, and $P = 0.239$ for rs1059394 C > T). As it shown in Figure 2, the three variants are in low to moderate linkage disequilibrium (LD) with one another; and the two adjacent SNPs (rs1059394 and rs2790) showed the strongest level of LD ($D' 0.89$ and $r^2 = 0.49$).

As shown in Table 2, the genotype distribution of *TYMS* rs16430 6bp > 0bp was significantly different between cases and controls ($P = 0.027$). Carrier of the rs16430 0bp variant allele had a significantly increased risk of breast cancer (6bp/0bp and 0bp/0bp + 6bp/0bp vs. 6bp/6bp; adjusted OR 1.39 and 1.37; 95% CI, 1.09–1.78 and 1.08–1.73; $P=0.009$ and 0.010 , $P_{trend}=0.026$, respectively), compared with carriers of rs16430 6bp/6bp. However, no association was observed for other SNPs (Table 2).

Stratified Analysis of *TYMS* Genotypes and Risk of Breast Cancer

We further performed stratified analysis to examine *TYMS* genotypes and risk of breast cancer by age, smoking status, and alcohol use. We found that the increased risk of breast cancer associated with the rs16430 0 bp variant allele was more evident among older women (>45 yr, adjusted OR=1.47, 95% CI = 1.06–2.03), never smokers (adjusted OR 1.67, 95% CI = 1.23–2.25), never-drinkers (adjusted OR=1.44, 95% CI = 1.01–2.05), and ER positive patients (adjusted OR=1.46, 95% CI=1.11–1.92). However, no effect was observed in other SNPs (rs2790 A > G and rs1059394 C > T), except the direction of the effect observed for the rs1059394T variant allele was more evident among ever smokers (adjusted OR=0.65, 95% CI=0.44–0.96, $P=0.032$), where a significantly decreased risk of breast cancer was observed (Table 3). In the case only analysis, we did not find any evidence of interaction between *TYMS* variants and tumor stage or PR status, except a borderline association between rs1059394T variant allele and breast cancer risk in ER positive patients (adjusted OR=1.17, 95% CI 0.99–2.18) (Supplement Table 1).

Because most of the significant findings were in the subgroup analysis, we calculated the FPRP values for all the observed significant associations. As shown in Table 4, when the assumption of prior probability was 0.25, the association with *TYMS* (rs16430) 0bp/0bp + 6bp/0bp genotypes was noteworthy for all subjects (FPRP=0.024), age >45 yr (FPRP=0.056), never smokers (FPRP=0.003), never drinkers (FPRP=0.118), and ER positive patients (FPRP=0.020). When the assumption of prior probability was 0.1, the association with *TYMS* (rs16430) 0bp/0bp 6bp/0bp genotypes was still noteworthy for all subjects (FPRP=0.069), age >45 yr (FPRP=0.152), never smokers (FPRP=0.008), and ER positive patients (FPRP=0.058).

DISCUSSION

In this case-control study, we investigated the association between *TYMS* polymorphisms/variants and risk of sporadic breast cancer in non-Hispanic white women who were 55 yr. We found that the rs16430 0 bp variant allele was significantly associated with increased risk of breast cancer. In the stratified analysis, this significant association was confined to subgroups of age >45 yr, never smokers, never drinkers and ER positive patients. Although multiple testing has been performed in the study, the results of FPRP indicated that our results were less likely to be false positives, which implies the functional SNPs in *TYMS* might be involved in the development of sporadic breast cancer with a high likelihood. The results are also consistent with the functional analyses of *TYMS* rs16430 variant as previously reported, in which the rs16430 variant 0bp allele led to significantly lower luciferase activity and lower *TYMS* mRNA expression, compared with the 6bp allele [10]. These data suggest that this miRNA-binding site variant (rs16430) may mediate the expression levels of *TYMS* and thus play a role in the etiology of breast cancer.

miRNAs regulate gene expression by the sequence-specific binding to target mRNA [38], but the binding affinity may be affected by SNPs/variants residing in the miRNA target sites, which may in turn lead to degradation of the mRNA or affect the miRNAs' ability to inhibit the mRNA translation into proteins [39]. Indeed, recent studies have demonstrated that SNPs at miRNA-binding sites are likely to affect expression of the miRNA target genes and thus may contribute to cancer susceptibility, including breast cancer [6,40–42]. For example, in the study of 697 patients with colorectal cancer and 624 healthy Caucasian population controls, Landi et al. [41] found that the variant alleles at the miRNA-binding site in the CD86 gene were associated with a significantly increased risk of colorectal cancer. In another study of 1,110 breast cancer cases and 1,097 cancer-free controls in a Chinese population, Song et al. [6] found that the *miR-502*-binding site SNP in the 3' UTR of *SET8* modulates the *SET8* expression and contributes to the early development of breast cancer.

Because *TYMS* plays an important role in folate metabolism and DNA repair, *TYMS* SNPs/variants have drawn special interest from laboratory, epidemiological and clinical investigators. Although epidemiological studies have evaluated the association between *TYMS* (rs16430) variant and breast cancer susceptibility, the published data are still inconclusive. For example, in an early case-control study of 575 breast cancer cases and 610 cancer-free controls in a Caucasian population, Justenhoven et al. [43] found that the risk associated with the rs16430 variant was not statistically significant. In a later Caucasian study of 150 breast cancer cases and 141 cancer free controls, Akisik and Dalay [8] reported that the rs16430 0 bp variant was associated with a significantly lower risk of breast cancer. In a recent study of 310 breast cancer cases and 290 cancer free controls in a Poland population, Jakubowska et al. [44] have failed to find an association between the rs16430 polymorphism and risk of breast cancer. In a study of 380 breast cancer cases and 662 cancer free controls in a Singapore Chinese population, Inoue et al. [45] found no association between *TYMS* rs16430 variant and breast cancer risk. In contrast, in another Chinese study of 432 breast cancer patients and 473 health controls, Zhai et al. [46] observed a decreased risk of breast cancer in individuals with the rs16430 6bp/6bp

genotype. In general, of the five published studies, two had a relatively small sample size [8,44], and the other three had a similar sample size to ours but had a wide range of onset ages [43,45,46], compared with our relatively young subjects (ages 55 yr) that may have the advantage to unravel genetic susceptibility. Finally, in a meta-analysis of five case-control studies (1,969 cases and 2,290 controls) of *TYMS* rs16430 variant and breast cancer, the authors concluded that this variant may be a genetic determinant for developing breast cancer in Asian populations; therefore, ethnic background should be carefully considered in further studies [9].

Compared with other studies, our present study is rather unique. We included relatively young (55 yr) non-Hispanic white women because genetic susceptibility is often characterized by an early age of onset, which is a potential risk factor for breast cancer. In the current study, We observed that frequencies of *TYMS* rs16430 6bp/6bp genotype (49.7%), 6bp/0bp genotype (43.8%) and 0bp/0bp genotype (6.5%) in 580 non-Hispanic controls were consistent with frequencies of 48.4%, 44.2%, and 7.3%, respectively, observed by Ulrich et al. [20] in 95 Caucasian controls and frequencies of 47.9%, 43.8% and 8.3%, respectively, observed in Justenhoven et al. [43] in 610 Germany controls. We found that the rs16430 0 bp variant allele was associated with significantly increased risk of breast cancer. Further stratified analysis showed that patients who were never smokers and never drinkers had a more pronounced increased risk of breast cancer when carrying the rs16430 0 bp variant allele, which is consistent with the concept that the susceptible individuals like to be young or may have been exposed to a low level of carcinogens for a shorter period of time, possibly due to the inherited poor DNA repair mechanism associated with *TYMS* variation. However, these results may also be influenced by the relatively small number of subjects in the stratified subgroups. Further studies with large sample sizes are needed to verify these findings.

Our results are also consistent with the functional analyses of *TYMS* rs16430 variant by Mandila et al., who demonstrated that the *TYMS* 3'-UTR construct containing the 6bp deletion had a high rate of mRNA degradation compared to the 6bp insertion construct, and the 6bp deletion (0bp) resulted in a ~70% decrease in luciferase activity and ~50% mRNA levels [10]. It is likely that the germline variants in the predicted binding sites in the target *TYMS* gene may have led to altered complementarity of nucleotides with the *miR-561* gene, thus leading to altered *TYMS* gene translation [47]. It is possible that the rs16430 variant affects mRNA stability or secondary mRNA structures, leading to altered protein levels or responses to up-regulation of this enzyme. In addition, altered miRNA binding to one site at *TYMS* mRNA will also change the secondary structure of the remaining binding sites, a dynamic process that could potentially impact other miRNA binding as well [38,39].

In the present study, the increased risk of breast cancer associated with the *TYMS* rs16430 0 bp variant allele was more evident in ER positive patients and women older than 45 yr than women in younger or reproductive age. Consistent with this result, Xie et al. demonstrated that ERα protein was required for hormone-mediated trans-activation of *TYMS* in MCF-7 human breast cancer cells [40]. Our result suggests that *TYMS* may have an interaction with female hormones in addition to regulating the expression of oncogenes. It is well known that female hormones have been implicated as a major etiological factor in the development of

breast cancer. Women's fecundity decline starts at age of 35, accelerates between 35 and 40 yr of age, and approaches zero by age 45 [48]. At that point, we could speculate that the change in hormonal status with aging may have affected the role of *TYMS* or its interaction with other oncogenes, attributing to increased cancer risk; however, this speculation needs to be tested in future studies.

This is the first study to our knowledge to focus on the effect of germline variants in the predicted miRNA binding sites in *TYMS* on breast cancer risk in a non-Hispanic white women population with a young age (< 55 yr). However, there are some limitations in our study. First, our study is a hospital-based case-control study with a relative small sample size, which might have a selection bias. Since the controls were selected in the hospital setting, they might not represent the general population. Second, we had very limited information about the intake of alcohol and folate, which may contribute to the variation in the observed cancer risk. Third, we did not have information about BRCA germline mutations in this study population, which may also contribute to the variation in the observed cancer risk. Fourth, we did not have reproductive information for the controls, which excluded these parameters from further analysis of risk association with *TYMS* polymorphisms. In addition, we found a significant correlation between smoking and drinking status ($P < 0.0001$) and between ER and smoking status ($P=0.033$), which may have led to the similar stratified results by the three factors. Further studies with large sample sizes are needed to verify these findings.

In conclusion, in this hospital-based case-control study of sporadic breast cancer, we found a significant association between the *TYMS* rs16430 0 bp variant allele and increased risk of breast cancer in non-Hispanic white women aged < 55. The increased risk was more evident in subgroups of age between 45–55 yr, never smokers, never drinkers and ER positive patients. Further validation of our findings in large population-based studies is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

TYMS	thymidylate synthase
OR	odd ratio
CI	confidence interval

dUMP	deoxyuridine-5'-monophosphate
dTMP	deoxythymidine-5'-monophosphate)
miRNAs	microRNAs
UTR	3' untranslated region
mRNAs	messenger RNAs
ER	estrogen receptor
PR	progesterone receptor
FPRP	false-positive report probability
LD	linkage disequilibrium

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Figure 1. Characterization and functional analysis of the *TYMS* 3' UTR and linkage disequilibrium (LD) display for the three SNPs of *TYMS*. (A) *TYMS* polymorphisms and their exact location within the *TYMS* 3' UTR. (B) Sequence alignment of miR-561 and its conserved binding site at position 663444 in *TYMS* 3' UTR (rs16430) based on the TargetScan analysis. (C) The disruption of rs16430-0bp at the 3'-UTR of *TYMS* mRNA will affect the interaction of *hsa-miR-561* with *TYMS* 3'UTR, resulting in elevated constitutive expression of *TYMS* mRNA translation. (D) RFLP-PCR genotyping method for the *TYMS* (rs16430) polymorphisms. Lanes 1–3, 5, 6, and 12 6bp/0bp; Lanes 4, 7, 8, and 11, 6bp/6bp; Lanes 9 and 10, 0bp/0bp.

Figure 2. Linkage disequilibrium display for the three variants of TYMS among non-Hispanic white controls. (A) The D' display and (B) The r^2 display. All polymorphisms were not in LD in the controls.

Table 1
Frequency Distribution of Selected Variables in Breast Cancer Cases and Controls

Variable	Cases (<i>n</i> =543)		Controls (<i>n</i> =580)		<i>P</i> ^a
	<i>n</i>	%	<i>n</i>	%	
Age (yr)					
<45	251	46.2	268	46.2	
>45	292	53.8	312	53.8	0.995
Race					
Non-Hispanic Whites	543	100	580	100	
Smoking status ^b					
Never	326	60.6	385	66.8	0.030
Ever	212	39.4	191	33.2	
Drinking status ^b					
Never	232	43.3	278	48.3	0.096
Ever	304	56.7	298	51.7	
Stage ^c					
0	72	13.3			
I	206	37.9			
II	189	34.8			
III	66	12.2			
IV	4	0.8			
ER ^c					
Negative	148	30.2			
Positive	342	69.8			
PR ^c					
Negative	202	41.3			
Positive	287	58.7			

^aTwo-sided chi-square test.

^bFive cases and four controls with missing smoking status and seven cases and four controls with missing alcohol use information.

^cSix patients with missing stage information, 53 patients with missing information on ER status and 54 n PR status.

Table 2Association Between the *TYMS* Genotypes Frequencies and Risk of Breast Cancer

Genotypes	Cases		Controls		Crude		Adjusted	
	n (%)	n (%)	n (%)	n (%)	OR (95% CI)	OR (95% CI) ^b	<i>P</i> ^a	<i>P</i> ^b
<i>TYMS</i> (rs2790)								
AA	339 (62.4)	348 (60.0)	0.557	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
AG	172 (31.7)	201 (34.7)		0.88 (0.68–1.13)	0.87 (0.68–1.13)	0.303		
GG	32 (5.9)	31 (5.3)		1.06 (0.63–1.78)	1.04 (0.61–1.74)	0.897		
<i>P</i> _{trend}			0.599					0.549
GG+AG	204 (37.6)	232 (40.0)	0.404	0.90 (0.71–1.15)	0.90 (0.70–1.14)	0.377		
<i>TYMS</i> (rs16430)								
6bp/6bp	227 (41.8)	288 (49.7)	0.027	1.00 (ref.)	1.00 (ref.)			
6bp/0bp	279 (51.4)	254 (43.8)		1.39 (1.09–1.78)	1.39 (1.09–1.78)	0.009		
0bp/0bp	37 (6.8)	38 (6.5)		1.24 (0.76–2.01)	1.22 (0.75–1.99)	0.421		
<i>P</i> _{trend}			0.026					0.032
0bp/0bp+6bp/0bp	316 (58.2)	292 (50.3)	0.008	1.37 (1.09–1.74)	1.37 (1.08–1.73)	0.010		
<i>TYMS</i> (rs1059394)								
CC	255 (47.0)	256 (44.1)	0.547	1.00 (ref.)	1.00 (ref.)			
CT	243 (44.7)	268 (46.2)		0.91 (0.71–1.16)	0.91 (0.70–1.15)	0.405		
TT	45 (8.3)	56 (9.7)		0.81 (0.53–1.24)	0.81 (0.53–1.24)	0.332		
<i>P</i> _{trend}			0.275					0.258
TT+CT	288 (53.0)	324 (55.9)	0.342	0.89 (0.71–1.13)	0.88 (0.70–1.12)	0.308		

^aTwo-sided chi-square test for genotype distribution.^bAdjusted for age, smoking status, and alcohol use in a logistic regression model.

Table 3

Stratified Analysis of *TYMS* Genotypes and Risk of Breast Cancer by Age, Smoking, and Alcohol Use

Variables	rs2790 (case/control)			rs16430 (case/control)			rs1059394 (case/control)					
	AA	GG+AG	Adjusted OR ^a (95%CI)	P ^a	6bp/6bp	0/0 + 6/0	Adjusted OR ^a (95% CI)	P ^a	CC	TT + CT	Adjusted OR ^a (95% CI)	P ^a
Age, yr												
45	166/158	85/110	0.73 (0.51–1.04)	0.082	116/140	135/128	1.27 (0.89–1.80)	0.184	128/120	123/148	0.76 (0.54–1.08)	0.131
>45	173/190	119/122	1.07 (0.77–1.49)	0.678	111/148	180/164	1.47 (1.06–2.03)	0.022	127/136	165/176	1.00 (0.72–1.39)	0.994
Smoking status												
Never	198/234	128/151	0.99 (0.73–1.35)	0.971	132/203	194/182	1.67 (1.23–2.25)	<0.001	114/175	182/210	1.05 (0.78–1.42)	0.74
Ever	139/112	73/79	0.75 (0.50–1.12)	0.153	94/83	118/108	0.96 (0.65–1.43)	0.857	110/79	102/112	0.65 (0.44–0.96)	0.032
Alcohol status												
Never	147/169	85/109	0.90 (0.63–1.29)	0.550	97/142	135/136	1.44(1.01–2.05)	0.043	108/129	124/149	1.00 (0.70–1.42)	0.996
Ever	189/177	115/121	0.90 (0.65–1.25)	0.519	127/144	177/154	1.31 (0.95–1.81)	0.105	145/125	159/173	0.80 (0.58–1.10)	0.167
Stage												
0	41/348	31/232	1.14(0.69–1.88)	0.603	29/288	43/292	1.42 (0.86–2.35)	0.168	36/256	50/324	0.79 (0.48–1.29)	0.338
I–II	249/348	146/232	0.94(0.74–1.21)	0.634	164/288	231/292	1.37 (1.07–1.74)	0.012	181/256	214/324	0.89 (0.70–1.13)	0.331
III–IV	46/348	24/232	0.93 (0.73–1.18)	0.537	31/288	39/292	1.36 (1.08–1.72)	0.009	35/256	35/324	0.88 (0.70–1.11)	0.281
ER												
(–)	97/348	51/232	0.78 (0.53–1.14)	0.025	66/288	82/292	1.24 (0.86–1.78)	0.259	78/256	70/324	0.71 (0.49–1.02)	0.061
(+)	206/348	136/232	0.99 (0.75–1.30)	0.93	137/288	205/292	1.46 (1.11–1.92)	0.006	149/256	193/324	1.01 (0.77–1.33)	0.921
PR												
(–)	129/348	73/232	0.83 (0.59–1.16)	0.281	83/288	119/292	1.44(1.04–2.00)	0.029	94/256	108/324	0.90 (0.65–1.24)	0.513
(+)	173/348	114/232	1.00 (0.74–1.33)	0.979	119/288	168/292	1.36 (1.02–1.82)	0.035	132/256	155/324	0.92 (0.69–1.23)	0.579

^a Adjusted for age, smoking status and alcohol status (the stratified factor in each stratum excluded).

Table 4
FPRP Values for Associations Between risk of Breast Cancer and Frequencies of *TYMS* rs16430 Genotypes

Genotype	Positive OR 95% CI ^a	<i>P</i> ^b	Statistical power ^c	Prior probability				
				0.25	0.1	0.01	0.001	0.0001
rs8126: 6bp/6bp > 0bp/0bp	6bp/6bp vs. 0bp/0bp + 6bp/0bp							
All subjects	1.37 (1.08–1.73)	0.008	0.999	0.024	0.069	0.448	0.891	0.988
Age >45	1.47 (1.06–2.03)	0.019	0.969	0.056	0.152	0.664	0.952	0.995
Never Smokers	1.67 (1.23–2.25)	0.001	0.882	0.003	0.008	0.077	0.458	0.894
Never drinkers	1.44 (1.01–2.05)	0.043	0.966	0.118	0.286	0.815	0.978	0.998
ER positive	1.46 (1.11–1.92)	0.007	0.988	0.020	0.058	0.404	0.872	0.986

^aThe crude OR.

^bThe omnibus chi-square test of the genotype frequency distributions.

^cCalculated using study subjects to detect an OR of 2.00 with the common genotype used as the reference.