Synergistic Effects of *STK15* Gene Polymorphisms and Endogenous Estrogen Exposure in the Risk of Breast Cancer

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Abstract

STK15 is a member of a family of serine/threonine kinases that act as key regulators of chromosome segregation and cytokinesis. Overexpression of the *STK15* gene leads to centrosome amplification, chromosomal instability, aneuploidy, and transformation. It has been reported that the 91T → A (Phe → Ile at codon 31) polymorphism in the *STK15* gene affects the function of this gene. We hypothesized that this polymorphism may interact with endogenous estrogen exposure in the risk of breast cancer and evaluated this hypothesis in a population-based, case-control study conducted among Chinese women in Shanghai. Genotyping assays were completed for 1,102 incident cases and 1,186 community controls. Participation and blood donation rates were over 90% and 80%, respectively. Elevated risks of breast cancer were found to be associated with the Phe/Ile [odds ratio (OR), 1.3; 95% confidence interval (CI), 1.0-1.7] and Ile/Ile (OR, 1.2; 95% CI, 0.9-1.6) genotypes at codon 31 of the *STK15* gene, although the ORs were not statistically significant. The risk associated with this polymorphism was modified by factors related to endogenous estrogen exposure, such as high body mass index (BMI), high waist-to-hip ratio, long duration of lifetime menstruation, or long duration of menstruation before first live birth. In particular, a statistically significant interaction was found between BMI and the *STK15* Phe[31]Ile polymorphism (P = 0.02) and a positive association with breast cancer risk for the *Ile* allele was found only among overweight (BMI ≥ 25 kg/m^2) women with adjusted ORs (95% CIs) of 3.3 (1.4-7.7) and 4.1 (1.7-9.8) associated with the Phe/Ile and Ile/Ile genotypes (P for trend <0.01), respectively. The findings from this study are consistent with the evidence from *in vitro* and *in vivo* experiments, implicating an etiologic role of the *STK15* gene in human breast cancer, and provide evidence for the modifying effects of genetic background on human cancer risk. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2065–70)

Introduction

STK15 (also known as BTAK, Aurora 2, and AIK1) is a member of the Aurora/Ipl1p family of mitotically regulated serine/threonine kinases that are key regulators of chromosome segregation and cytokinesis (1-3). A wealth of data indicates that overexpression of the *STK15* gene leads to centrosome amplification, chromosomal instability, aneuploidy, and transformation (3-7) and has been detected in a variety of human cancers, including breast cancer (4, 6, 8-12). Breast cancer is a hormone-related cancer, and sex hormones increase breast cancer risk by causing proliferation of breast epithelial cells. Replication errors and genetic damage during cell division, if not corrected, may lead to breast cancer (13, 14). A recent study found that the *STK15* gene is expressed predominantly in cells or tissues with proliferative activity (15). Furthermore, it has been shown that the expression level of the *STK15* gene is induced substantially after estradiol treatment (16). Therefore, it is conceivable that STK15, a cell cycle regulator, may interact with estrogen, a cell proliferation stimulator, in the pathogenesis of breast cancer. We investigated this hypothesis by evaluating the association between breast cancer risk and a common functional polymorphism (91T → A, Phe[31]Ile) in the *STK15* gene and examine further whether this association may be modified by factors related to estrogen exposure in an epidemiologic study (3).

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Materials and Methods

Study Participants and Data Collection. Included in this study were subjects recruited from 1996 to 1998 in the Shanghai Breast Cancer Study. Detailed study methods have been published elsewhere (17). Relevant

committees for the use of human subjects in research approved the study protocol. In brief, this study consisted of 1,459 incident breast cancer cases diagnosed in women ages 25 to 64 years and 1,556 age frequency–matched community controls. Cancer cases were identified through a rapid case ascertainment system, supplemented by the population-based Shanghai Cancer Registry, which has virtually complete ascertainment of all incident cancer cases diagnosed among residents in urban Shanghai (18). A total of 1,602 eligible breast cancer cases were identified during the study period, of which 1,459 (91.1%) cases completed in-person interviews. Cancer diagnoses for all patients were reviewed and confirmed by two senior pathologists. Controls were randomly selected from the general population of Shanghai using the Shanghai Resident Registry, a population registry containing demographic information for all adult residents of urban Shanghai. The inclusion criteria for controls were identical to those for cases with the exception of a breast cancer diagnosis. Of the 1,724 eligible women, 1,556 (90.3%) completed in-person interviews.

A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. All participants were measured for their current weight and circumferences of the waist and hips. Of those who completed the in-person interviews, 1,193 cases (82%) and 1,310 controls (84.2%) donated a blood sample. All of the specimens were collected in the morning before any meals. These samples were processed on the same day, typically within 6 hours of sample collection, and stored at −70°C until relevant bioassays were carried out.

Laboratory Genotyping Methods. *STK15* genotyping was done using the Taqman 5′ Nuclease Assay (Applied Biosystems, Foster City, CA; ref. 19). The primers and probes were purchased from ABI Assay-by-Design services. The primers and probes for Phe[31]Ile polymorphism were forward: 5′-TGGAGGTCCAAAACGTGTTCTC-3′ and reverse: 5′-CTGCCCACTATTTACAGGTAATGGA-3′; probes were VIC-ACTCAGCAAATTC CTT for the *Ile* allele and FAM-ACTCAGCAATTTCCTT for the *Phe* allele. In addition to Phe[31]Ile polymorphism, we also evaluated a single nucleotide polymorphism at codon 57 using the primers forward: 5′-GGGTCTT GTGTCCT-TCAAATTCTTC-3′ and reverse: 5′-CGGC TTGTGACT-GGAGACA-3′. Probes were VIC-CAG CGCGTTCCTT for the *Val* allele and FAM-CAGCGCATTCCTT for the *Ile* allele. PCR was done in a total volume of 5 μL containing 2.5 ng DNA, 1× Taqman Universal PCR Master Mix, 900 nmol/L each primer, and 200 nmol/L each probe. The thermal cycling conditions were as follows: 50°C for 2 minutes and 95°C for 10 minutes to activate AmpErase uracil *N*-glycosylase and AmpliTaq Gold enzyme, respectively, followed by 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. The fluorescence level was measured with ABI PRISM 7900HT sequence detector (Applied Biosystems). Allele frequencies were determined by ABI SDS software. Among those who provided a blood sample, genotyping data were obtained from 1,102 (92%) cases and 1,186 (90%) controls for the Phe[31]Ile polymorphism and 1,102 (92%) cases and 1,188 (91%) controls for the Val[57]Ile polymorphism. The major reasons for incomplete genotyping were insufficient DNA used in the assay and unsuccessful PCR amplification.

The laboratory staff were blind to the identity of the subjects. Quality control samples were included in the genotyping assays. Each 386-well plate of genomic DNA contained multiple controls, including four water blanks, eight samples of CEPH 1347-02, eight unblinded quality control samples, and eight blinded quality control samples. The duplicated blind quality control samples were distributed across separate 384-well plates. *STK15* genotypes determined for the duplicated quality control samples were in complete agreement.

Statistical Analysis. χ^2 statistics were used to evaluate case-control differences in the distribution of genotypes. Multivariate logistic regression models were used to estimate the odds ratio (OR) and their 95% confidence interval (95% CI) as a measure of the strength of the association. χ^2 goodness-of-fit test was used for testing *STK15* genotypes for Hardy-Weinberg equilibrium. Linkage disequilibrium between Phe[31]Ile and Val[57]Ile polymorphisms was tested using R^2 values (20). Haplotype frequencies were estimated via expectation-maximization algorithms (21). Tests for trend across tertiles were done in logistic regression models by assigning the score j to the jth level of the variable selected. Stratified analyses by indicators of endogenous estrogen exposure were conducted to evaluate the potential modifying effects of these variables on the association between *STK15* genotypes and breast cancer risk. Multiplicative interactions were formally evaluated in logistic regression models by likelihood ratio tests. $P < 0.05$ (two-sided probability) was interpreted as statistically significant.

Results

Selected demographic characteristics and major risk factors are compared for cases and controls in Table 1. Cases and controls were similar in age. With the exception of a family history of breast cancer, statistically significant associations were observed for all major risk factors of breast cancer. More cases than controls had a family history of breast cancer, although the difference was not statistically significant, due to a low prevalence of positive breast cancer family history in this population. There was no appreciable difference between cases included in the genotyping study and the whole study (data not shown).

The allele and genotype distributions for the two common polymorphisms in the *STK15* gene are presented in Table 2. The distribution of genotypes for these two polymorphisms is consistent with the Hardy-Weinberg equilibrium for both cases and controls. A nonsignificantly elevated risk was associated with the *Ile31* allele. One striking observation was that the frequency of the Ile/Ile genotype in this Chinese population (>40%) was much higher than that reported in a Caucasian population (~6%; ref. 3). There was no apparent difference in allele frequency or genotype of the polymorphism at codon 57. When the two STK15 polymorphisms were analyzed jointly, women with both

Table 1. Comparison of cases and controls by selected descriptive characteristics, Shanghai Breast Cancer Study, 1996-1998

Subject characteristic	Cases (n = 1,132)	Controls (n = 1,222)	P*
Age (y), mean ± SD	47.6 ± 8.0	47.2 ± 8.7	0.20
Education (%)			
Elementary school or below	12.3	14.6	
Middle or high school	75.7	75.4	
College or above	12.0	10.1	0.11
Breast cancer in first-degree relative (%)	3.4	2.4	0.12
Ever had breast fibroadenoma (%)	9.7	5.2	<0.01
Age at menarche (y)	14.5 ± 1.6	14.7 ± 1.7	<0.01
Age at first live birth (y), mean ± SD	26.8 ± 4.1	26.2 ± 3.8	<0.01
Postmenopausal (%)	33.2	36.2	0.13
Age at menopause (y), mean ± SD	48.2 ± 4.6	47.5 ± 5.0	0.04
Physically active past 10 y (%)	19.2	25.7	<0.01
BMI (kg/m^2), mean ± SD	23.5 ± 3.4	23.2 ± 3.4	0.03
WHR, mean ± SD	0.81 ± 0.06	0.80 ± 0.06	<0.01

*For χ^2 test (categorical variables) or t test (continuous variables).

Ile31 and *Ile57* alleles were at an ~40% increased risk of breast cancer (OR, 1.4; 95% CI, 1.0-2.1) compared with those who were homozygous for both *Phe31* and *Val57* alleles, although the results remained statistically nonsignificant (data not shown). This positive association for the joint genotype appeared among both premenopausal and postmenopausal women with ORs (95% CIs) of 1.3 (0.8-2.1) and 1.8 (0.9-3.4), respectively. The two polymorphisms are in modest linkage disequilibrium (R^2 = 0.30; P < 0.0001). However, none of the four derived common haplotypes was associated with a statistically increased risk of breast cancer (data not shown).

Table 3 shows a more detailed analysis of the association between the Phe[31]Ile polymorphism and breast cancer risk, stratified by body mass index (BMI), waist-to-hip ratio (WHR), years of lifetime menstruation, or years of menstruation before first live birth, all of which are indicators of endogenous estrogen exposures. The positive association between Phe/Ile and Ile/Ile genotypes and breast cancer risk was primarily seen among women with a high BMI or WHR, particularly among postmenopausal women. Overweight postmenopausal women had more than a 4-fold increased risk of developing breast cancer, if they carried the Ile/Ile genotype, compared with those with the Phe/Phe genotype (OR, 4.1; 95% CI, 1.7-9.8). This pattern of association suggests an interaction and the tests for multiplicative interaction were statistically significant for BMI (P for

Table 2. *STK15* allele and genotype frequencies, unadjusted and adjusted ORs for breast cancer, Shanghai Breast Cancer Study, 1996-1998

STK15		Cases, n (%)	Controls, n (%)	OR* (95% CI)	OR† (95% CI)
Genotype frequencies (%)					
Phe[31]Ile					
Phe/Phe		121 (11.0)	149 (12.6)	1.0	1.0
Phe/Ile		491 (44.6)	503 (42.4)	1.2 (0.9-1.6)	1.3 (1.0-1.7)
Ile/Ile		490 (44.5)	534 (45.0)	1.1 (0.9-1.5)	1.2 (0.9-1.6)
P for trend				0.73	0.66
Val[57]Ile					
Val/Val		805 (73.0)	908 (76.1)	1.0	1.0
Val/Ile		281 (25.5)	263 (22.0)	1.2 (1.0-1.5)	1.2 (1.0-1.5)
Ile/Ile		16 (1.4)	17 (1.6)	0.8 (0.4-1.6)	0.8 (0.4-1.6)
P for trend				0.19	0.20
Combined					
Codon 31	Codon 57				
Phe/Phe	Val/Val	45 (4.2)	66 (5.7)	1.0	1.0
	Val/Ile or Ile/Ile	72 (6.7)	76 (6.6)	1.2 (0.8-1.9)	1.2 (0.7-1.9)
	Val/Ile	56 (5.2)	56 (4.8)		
	Ile/Ile	16 (1.5)	20 (1.7)		
Phe/Ile	Val/Val	261 (24.3)	290 (25.1)	1.1 (0.8-1.6)	1.2 (0.8-1.7)
	Val/Ile or Ile/Ile	215 (20.1)	198 (17.1)	1.4 (1.0-2.0)	1.4 (1.0-2.1)
	Val/Ile	215 (20.1)	196 (16.9)		
	Ile/Ile	0 (0.0)	2 (0.2)		
Ile/Ile	Val/Val	476 (44.4)	525 (454)	1.1 (0.8-1.6)	1.2 (0.8-1.7)
	Val/Ile or Ile/Ile	3 (0.3)	2 (0.2)	1.9 (0.3-11.4)	1.2 (0.2-9.2)
	Val/Ile	3 (0.3)	2 (0.2)		
	Ile/Ile	0 (0.0)	0 (0.0)		

NOTE: The frequencies of the *Ile31* allele were 67% in cases and 66% in controls (P = 0.71), and frequencies of the *Ile57* allele were 14% in cases and 13% in controls (P = 0.19).

*Adjusted for age only.

†Adjusted for age, personal history of fibroadenoma, WHR, age at first live birth, age at menarche, physical activity, and menopausal status.

Table 3. Association of the *STK15* codon 31 genotype with breast cancer, stratified by selected life-style and reproductive factors, Shanghai Breast Cancer Study, 1996-1998

Variables	$Phe^{31}Ile$-Phe/Phe		$Phe^{31}Ile$-Phe/Ile		$Phe^{31}Ile$-Ile/Ile		*P* for trend
	Cases/controls	OR	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	
All subjects							
BMI*							
<25	95/101	1.0	325/363	1.0 (0.7-1.4)	345/399	0.9 (0.7-1.3)	0.62
≥25	26/48	1.0	166/140	2.3 (1.3-4.0)	145/135	2.2 (1.2-3.9)	0.05
P for interaction = 0.017							
WHR†							
<0.835	89/102	1.0	351/380	1.1 (0.8-1.5)	343/404	1.0 (0.7-1.3)	0.50
≥0.835	32/47	1.0	140/123	1.9 (1.11-3.28)	147/130	2.0 (1.2-3.5)	0.03
P for interaction = 0.06							
Years of menstruation‡							
≤27	47/71	1.0	235/290	1.3 (0.8-1.9)	231/292	1.2 (0.8-1.9)	0.54
>27	74/78	1.0	256/213	1.2 (0.9-1.8)	259/242	1.1 (0.8-1.6)	0.81
P for interaction = 0.92							
Years of menstruation before first live birth‡,§							
≤11	56/71	1.0	234/288	1.0 (0.7-1.5)	236/286	1.0 (0.7-1.5)	0.51
>11	65/78	1.0	257/215	1.5 (1.0-2.2)	254/248	1.3 (0.9-1.9)	0.57
P for interaction = 0.16							
Postmenopausal women							
BMI‖							
<25	21/28	1.0	93/105	1.2 (0.6-2.3)	95/131	0.9 (0.5-1.7)	0.32
≥25	10/29	1.0	68/66	3.3 (1.4-7.7)	78/67	4.1 (1.7-9.8)	<0.01
P for interaction = 0.019							
WHR¶							
<0.835	20/35	1.0	101/114	1.5 (0.8-2.9)	106/126	1.3 (0.7-2.4)	0.86
≥0.835	11/22	1.0	60/57	2.4 (1.0-5.7)	67/72	2.4 (1.0-5.8)	0.13
P for interaction = 0.58							
Years of menstruation‡							
≤29	17/24	1.0	78/104	1.2 (0.6-2.4)	81/106	1.3 (0.6-2.6)	0.53
>29	14/33	1.0	83/67	2.9 (1.4-5.9)	92/92	2.3 (1.1-4.7)	0.15
P for interaction = 0.09							
Years of menstruation before first live birth‡,§							
≤9	18/30	1.0	73/104	1.1 (0.6-2.3)	82/105	1.2 (0.6-2.4)	0.51
>9	13/27	1.0	88/67	2.9 (1.3-6.1)	91/93	2.2 (1.0-4.6)	0.33
P for interaction = 0.16							

*Adjusted for age, personal history of fibroadenoma, years of menstruation, age at first live birth, physical activity and WHR.
†Adjusted for age, personal history of fibroadenoma, years of menstruation, age at first live birth, physical activity and BMI.
‡Adjusted for age, personal history of fibroadenoma, physical activity, and WHR.
§Years of menstruation = age at menopause or age at interview for premenopausal women − age at menarche.
‖Adjusted for age, personal history of fibroadenoma, age at menarche, age at menopause, age at first live birth, physical activity and WHR.
¶Adjusted for age, personal history of fibroadenoma, age at menarche, age at menopause, age at first live birth, physical activity and BMI.

interaction = 0.02 both for all subjects combined and postmenopausal women). In addition, among postmenopausal women, the association of the $Phe^{31}Ile$ polymorphism with breast cancer risk was stronger among those with a longer duration of menstruation (*P* for interaction = 0.15). Similar analyses were done for the polymorphism at codon 57 (data not shown) and no appreciable difference was observed. The number of subjects homozygous for the *Ile57* allele, however, was small.

Discussion

We found that the *Ile31* allele of the *STK15* gene was associated with an increased risk of breast cancer, particularly among overweight postmenopausal women in the Shanghai Breast Cancer Study. This positive association also seems to be modified by other indicators of high or long-term endogenous estrogen exposure. These findings are new and suggest an important role for *STK* polymorphism in hormone-related cancers, such as breast cancer.

There were few methodologic limitations in this population-based study. The participation rate was high (>90%), minimizing potential selection bias. Although not all study participants (17%) donated a blood sample and not all DNA samples (8%) were successfully genotyped, we found that those participants with genotyping data were comparable for all major known risk factors and demographic characteristics with all subjects.

Differential recall biases, another major limitation in most case-control studies, are also not a major concern in this study because the accuracy of genotyping should not be affected by case-control status. Extensive information on anthropometrics and life-style factors were collected in the study for evaluating confounding factors and effect modifiers. In addition, Chinese women living in Shanghai have relatively homogeneous ethnic backgrounds, >98% of them are classified into a single ethnic group (Han Chinese). Therefore, the potential confounding effect of ethnicity for genotyping data is not a major concern.

The *STK15* gene is identified in chromosome 20q13.2, and the amplification of 20q is found in a variety of cancers, including hormone-related cancers, such as breast, prostate, ovarian, and colon cancers (22). An increased copy number of 20q13.2 is observed in ~12% to 18% of primary breast tumors and 40% of breast tumor cell lines (6, 23) and is associated with aggressive tumor behavior, cellular immortalization, and genomic instability (3, 7). Berry et al. (24) identified a susceptibility locus in the 20q13 region in a genome-wide search among 162 North American families with three or more members diagnosed with prostate cancer. The most significant evidence for linkage appeared among 46 families without male to male transmission, with an estimated 56% of the families linked (24). Collins et al. (25) evaluated the expression of five candidate genes at 20q13.2, a highly amplified region in breast cancer tissues and breast cancer cell lines. Of the *ZNF217*, *ZNF218*, *NABC1*, *PIC1L*, and *CYP24* genes evaluated, only *ZNF217* satisfied their criteria for an oncogene involved in breast cancer. Subsequent investigations showed that *CYP24* is also a candidate oncogene in this region (26). In addition to these two candidate genes, immunohistochemical analyses by Tanaka et al. (1) showed that overexpression of the *STK15* gene was detected in 94% of invasive ductal adenocarcinomas of the breast.

In an early study conducted by some members of the research group, a series of polymorphisms in the *STK15*, *ZNF217*, and *CYP24A1* genes were evaluated in relation to breast cancer (3). These studies provided suggestive evidence for linkage of the *STK15 Ile31* allele with breast cancer susceptibility in a Caucasian population, but the results did not reach statistical significance at that stage (3). Further studies with additional samples from this Caucasian population are in progress. Subsequently, this *Ile31* allele was found to be preferentially amplified in colon cancers and associated with the degree of aneuploidy in human colon tumors (3). Furthermore, the *Ile31* allele had a greater potency than the *Phe31* allele in inducing cell growth and tumorigenicity in mice (3). These findings, together with those from the current study, suggest that the *Ile31* allele may be a genetic susceptibility factor for breast cancer.

Our findings for a potential interaction of $Phe^{31}Ile$ polymorphism with BMI and other indicators of estrogen exposure are interesting. After menopause, adipose tissue is the major site for estrogen synthesis and women with a high BMI have an elevated level of endogenous estrogens (27). Moreover, either body weight (measured by BMI) or central obesity (measured by WHR) has also been linked to an elevated level of insulin and insulin-like growth factors (28, 29). Estrogens were shown to work synergistically with insulin-like growth factors in growth stimulation and might, in turn, promote mammary carcinogenesis (30). As with studies conducted elsewhere, we found in the Shanghai Breast Cancer Study that BMI was associated with an increased risk of postmenopausal breast cancer, whereas WHR was related positively to both premenopausal and postmenopausal breast cancer (31). Our findings also suggest that the association between STK15 polymorphisms and breast cancer risk could be modified by years of lifetime menstruation and years of menstruation before first live birth. These two indicators measure the duration of endogenous estrogen exposure and the latter also measures estrogen exposure during a particularly susceptible period in a woman's life cycle, as the number of undifferentiated/vulnerable breast cells is reduced substantially after the first pregnancy (32, 33). Several human and *in vitro* studies have supported the potential interaction between STK15 and estrogen exposure. The degree of overexpression of STK15 found in invasive ductal adenocarcinomas of the breast seems to be higher than that in non-hormone-related cancers, such as bladder, pancreatic, and stomach cancers (34-36). These results, however, were derived from different laboratories and need to be confirmed in future studies conducted in the same laboratory under the same conditions. In a very recent study, Hodges et al. (16) found that the expression level of the *STK15* gene was induced over three times after estradiol treatment in MCF-7 cell lines, whereas Tanner et al. (37) found in an earlier study that amplification of 20q13 is highly associated with a high S-phase fraction, an indicator of proliferative activity (22). Interestingly, amplification of the *STK15* gene is only detected in ~12% to 18% of breast tumors, whereas overexpression is seen in >90% of cases (1). This contrasts with the situation seen in, for example, colon tumors, where the proportion of cases showing *STK15* amplification is much higher (4). It is tempting to speculate that, in breast tissue, selection pressure for *STK15* amplification may be lower because the gene is already up-regulated by estrogen exposure. Another interesting finding is that the frequency of homozygotes for the risk genotype (Ile/Ile) is 7-fold higher in the Chinese population than in Caucasians (~40% versus 6%, respectively). This is unexpected because Chinese women have a lower incidence rate of breast cancer compared with their counterparts in Western countries (18, 38). Breast cancer, however, has a complex etiology, involving multiple genetic and environmental factors. The fact that breast cancer incidence is not highly elevated in the Chinese women is presumably due to the buffering effects of environmental factors or other polymorphisms in this genetic background. It is well known from animal models that genetic interaction between low-penetrance predisposing alleles play an important role in determining cancer risk (39). Future comparisons of Caucasian and Asian populations may therefore provide a fruitful avenue for the detection of such interactions from studies of cancer risk conferred by combinations of polymorphisms in candidate genes.

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