

Genetic Polymorphisms of *SULT1A1* and *SULT1E1* and the Risk and Survival of Breast Cancer

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Abstract

We examined whether common single nucleotide polymorphisms (SNP) in *SULT1A1* (c.779G>A, *14A>G, and *85C>T) and *SULT1E1* (IVS1-447C>A, IVS4-1653T>C, and *959G>A) genes influenced the risk and survival of breast cancer. Our study population consisted of 989 histologically confirmed sporadic breast cancer patients and 1,054 controls without history of cancer recruited from three teaching hospitals in Seoul. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by logistic regression model. In the survival analysis for 529 breast cancer patients with completed treatments, the hazard ratios (HR) were calculated with Cox proportional hazard model. Women with the *SULT1E1* *959 GA/AA genotype had a moderately decreased breast cancer risk compared with those with the GG genotypes (OR, 0.8; 95% CI, 0.70-1.00). When the haplotypes were considered, the homozygous *959 AA genotype together with the IVS4-1653 T>C

base change (CTA-CCA haplotype) was associated with halved breast cancer risk (OR, 0.5; 95% CI, 0.24-0.88) compared with the wild type CTG-CTG haplotype. No other significant overall association was observed between the *SULT1A1* and *SULT1E1* SNPs nor haplotypes and breast cancer risk. When stratified by survival, patients with the *SULT1E1* IVS4-1653 TC/CC genotypes showed a >3-fold risk of recurrence (HR, 3.2; 95% CI, 1.39-7.48) compared with those with the TT genotype. Moreover, when the haplotypes were considered, the *SULT1E1* *959 G>A base change together with the IVS4-1653 T>C base change (CTG-CCA haplotype) was associated with a >4-fold risk of breast cancer (OR, 4.2; 95% CI, 1.15-15.15). These findings suggest that genetic polymorphisms of *SULT1E1* are associated with increased risk and a disease free survival of breast cancer in Korean women. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1090-5)

Introduction

The involvement of estrogens in breast carcinogenesis has been appreciated for a number of years. In addition, estrogen stimulation is an important factor in human breast cancer cell growth and development via estrogen receptor (ER)-mediated cellular events. The role of catechol estrogens as genotoxic chemical procarcinogens, independent of ER mediation, has also recently been recognized (1).

Estrogen conjugation is a major route of estrogen metabolism (1). Because conjugated estrogens are not appreciable ligands for the ERs, they do not promote ER-mediated mitogenicity. The most abundant circulating estrogen exists as a sulfate conjugate. The conjugation reaction is catalyzed by human sulfotransferases (SULT; ref. 2). Therefore, genetically determined individual variation in the SULT-mediated sulfate conjugation capacity may contribute to the estrogen-dependent carcinogenesis (3).

The most important SULTs from an estrogen-dependent carcinogenesis point of view are *SULT1A1* and *SULT1E1* sulfonating estrone, estradiol, and other steroid compounds. *SULT1A1* has been shown to be highly expressed in breast

cancer cell lines (4). A common single nucleotide polymorphism (SNP) has been observed in the *SULT1A1* gene (c.779G>A) that results in an arginine-to-histidine amino acid change in codon 213 that significantly influence the enzymatic activity; individuals with two His²¹³ alleles had only 15% of the *SULT1A1* activity compared with the carriers of the Arg²¹³ allele (5). Some rather small previous studies (103-444 cases) involving different ethnic study populations that have explored the potential association between the *SULT1A1* c.779G>A polymorphism and breast cancer risk have shown inconsistent results (6-9). A number of studies with conflicting outcomes have also been conducted on *SULT1A1* c.779G>A polymorphism and cancers of lung, colon, prostate, bladder, esophagus, and urinary tract (10-21).

SULT1E1 exhibits the highest affinity for estrogens among SULTs (22) indicating that it is active at physiologically significant concentrations of estrogens (23). Moreover, *SULT1E1* is highly expressed in normal human mammary epithelial cells (4) and might play an important role in estrogen-driven breast cancer development. Although *SULT1E1* seems rarely expressed in breast cancer cell lines (4), its expression has been detected in human breast carcinomas, which in turn was associated with a decreased risk of recurrence or improved prognosis of breast cancer (24).

To date, >20 SNPs have been found in the *SULT1E1* gene (25, 26). These polymorphisms include functionally different but rare polymorphisms in Caucasians, Africans, and Japanese. However, to our knowledge, no studies on the association between *SULT1E1* SNPs and breast cancer risk or survival have been reported to date.

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We examined the potential association between the *SULT1A1* and *SULT1E1* SNPs, for which the variant allele frequencies based on National Center for Biotechnology Information-SNP (<http://www.ncbi.nlm.nih.gov/SNP>) and JSNP (<http://snp.ims.u-tokyo.ac.jp>) databases were >10%, and breast cancer risk in a large case-control study in Korean. Association between these SNPs and breast cancer survival was also evaluated.

Materials and Methods

Study Subjects. The cases consisted of a consecutive series of breast cancer patients admitted to three teaching hospitals located in Seoul, Korea (SNUH, Borame, and Asan) between 1995 and 2003. The control subjects consisted of noncancer patients admitted to the same hospitals as the cases and of healthy women who participated in the community health screening program provided by a teaching hospital located in Seoul (EWUMC) in 2003. The study design was approved by the Committee on Human Research of Seoul National University Hospital, and the subjects provided their informed consents before participation in the study.

Eligible subjects included 1,007 histologically confirmed incident breast cancer patients and 1,136 cancer-free controls from whom the DNA samples were available. After exclusion of subjects with previous history of cancer or previous history of hysterectomy and oophorectomy, the final study population comprised 989 breast cancer cases and 1,054 cancer-free controls; the control group consisted of 471 healthy women and 710 hospital controls with noncancerous diseases including infection or stone of gall bladder/bile duct (26%), benign breast disease (17%), acute appendicitis (14%), hemorrhoid (8%), hernia/perforation (7%), lipoma (2%), and others (26%). Approximately 20% of cases and 14% of controls approached were excluded from the final study groups because of refusal to participate, failure to interview, and no blood collection. The criteria of subject selection and details of data collection on lifestyle have been described elsewhere (27). Information on demographic characteristics; education; marital status; family history of breast cancer in the first- and second-degree relatives; reproductive and menstrual factors; and lifestyle habits, including smoking (smoked at least 400 cigarettes/lifetime), alcohol consumption (<1/mo, ≤1-3/mo, ≥1/wk), oral contraceptive (OC) use, and hormone replacement therapy (HRT), were collected by trained interviewers using a structured questionnaire. Risk factors and genotype frequencies were not different between benign breast disease and other diseases among hospital controls and between the hospital controls and healthy controls (data not shown). Thus, the final statistical analyses were done by adjusting for all significant covariates identified from the initial analysis.

The subjects for survival analysis included 529 consecutive patients who underwent surgery for primary breast cancer between 1997 and 2002. Patients were followed up for disease-free survival from time of surgery until the first clinically recognized evidence of local or distant recurrence, death, or loss to follow-up. The distribution of follow-up for patients still alive at the time of analysis or loss to follow-up ranged from 3.8 to 73.3 months, with a median of 13.6 months. During the respective follow-up periods, 23 patients (4.3%) developed cancer relapse and four patients (0.8%) died. The clinicopathologic characteristics of the patients including age at onset, disease stage (tumor-node-metastasis stage), cancer treatments, and ER/progesterone receptor (PR) status were abstracted from medical records using a standard protocol.

Genotyping. Three *SULT1A1* and four *SULT1E1* SNPs, exhibiting reported variant allele frequencies higher than 10%, were selected from the National Center for Biotechnology Information-SNP (<http://www.ncbi.nlm.nih.gov/SNP>) and JSNP (<http://snp.ims.u-tokyo.ac.jp>) databases. Of these seven SNPs, six were successfully genotyped in the present study population. The *SULT1A1*¹⁰ (NT_010393, NM_177536.1) c.779G>A (R213H), *14A>G (rs6839, in the 3' flanking region), and *85C>T (rs1042157, in the 3' flanking region) and *SULT1E1*¹⁰ (NT_077444, NM_005420.2) IVS1-447C>A (rs3775778, intron 1), IVS4-1653T>C (rs3775775, intron 4), and *959G>A (rs3786599, in the 3' flanking region) were determined by the 5'-nuclease assay (Taqman). PCR primers used in the assays and Taqman Minor Groove Binder (MGB) probes labeled with dyes (FAM or VIC) at the 5'-end are listed in Appendix 1. Details of the genotyping protocol were described elsewhere (27).

Genotyping analyses were done blindly to the case-control status. The repeatability tests were conducted for five samples with 10 repeats randomly placed in the 384-well plates. Out of 250 reactions (50 samples for five loci), five results could not be determined and four discordant scorings emerged. A 94.0% concordance rate was thus achieved. When the undermined genotypes were excluded the concordance rate was 95.9%.

Statistical Analysis. The genotype frequencies were tested against Hardy-Weinberg equilibrium (HWE) by the χ^2 test. The associations between SNPs or haplotypes of *SULT1A1* or *SULT1E1* and breast cancer risk were estimated as odds ratios (OR) and 95% confidence intervals (95% CI) by unconditional logistic regression model adjusting for age (years), family history of breast cancer in first- and second-degree relatives (yes/no), and lifetime estrogen exposure (years; presenting the number of years of exposure to menstrual cycles, which is calculated according to the age at menarche and age at interview for premenopausal women and age at menarche and age at menopause for postmenopausal women). Linear increase in the risk with environmental exposure or genotype was evaluated by the likelihood ratio test. The product variable between genotypes and environmental exposure [genotype] \times [exposure] was added in the logistic model when evaluating the multiplicative interactive effect of *SULT1A1* or *SULT1E1* genotypes and environmental exposure on breast cancer risk.

After excluding the missing data with at least one out of each three polymorphic sites of *SULT1A1* or *SULT1E1*, the individual haplotypes were estimated from the genotype data by the Bayesian method using PHASE program (ver. 2.0.2; ref. 29). Pairwise linkage disequilibrium between any two alleles out of three polymorphic sites was estimated as relative disequilibrium (D') from estimated haplotype data using the following equations: (a) $D = pAB - pApB$; (b) $D' = D / D_{\max}$, where $D_{\max} = \min(pApb, papB)$ if $D \geq 0$; and (c) $D' = -D / D_{\min}$, where $D_{\min} = \max(-pApB, -papB)$ if $D < 0$, and the statistical significance was evaluated by the Fisher's exact test. All of the alleles in the three loci of *SULT1A1* and *SULT1E1* were found to be in strong linkage disequilibrium ($D' > 0.75$, $P < 0.001$ in *SULT1A1*; $D' > 0.73$, $P < 0.001$ in *SULT1E1*). The distributions of the haplotypes in the cases and controls were compared by χ^2 test. In diplotype (combination of haplotype) analysis, the diplotype consisting of the most common haplotypes (GAC for *SULT1A1* and CTG for *SULT1E1*) was used as the reference. The risk of breast cancer was estimated for each diplotype compared with the common diplotype after adjusting for other covariates.

¹⁰Nonmenclature followed by den Dunnen and Antonarakis (28).

Table 1. Selected characteristics for 989 breast cancer cases and 1,054 control subjects (adjusted OR)

	Case (%), n = 989	Control (%)			OR (95% CI)*, P*
		Hospital	Community	All	
		n = 583	n = 471	n = 1,054	
Age (mean ± SD)	47.0 ± 10.1	47.5 ± 13.4	51.2 ± 9.9	49.2 ± 12.1 [†]	<0.001
Education					
Under high school	312 (31.6)	233 (40.2)	172 (37.2)	405 (38.9)	1.0 (reference)
At/over high school	674 (68.4)	347 (59.8)	290 (62.8)	637 (61.1)	1.1 (0.85-1.31)
Fx of BrCa [‡]					
No	922 (93.2)	568 (97.4)	456 (96.8)	1024 (97.2)	1.0 (reference)
Yes	67 (6.8)	15 (2.6)	15 (3.2)	30 (2.9)	2.5 (1.60-4.02)
LEE (per 5 y)	29.0 ± 6.2	27.0 ± 7.6	31.1 ± 6.1	28.8 ± 7.3 [†]	1.3 (1.18-1.45)
Oral contraceptive use					
No	840 (85.0)	468 (88.0)	402 (85.7)	870 (86.9)	1.0 (reference)
Yes	148 (15.0)	64 (12.0)	67 (14.3)	131 (13.1)	1.2 (0.95-1.59)
HRT					
No	769 (88.6)	372 (95.9)	438 (93.0)	810 (94.3)	1.0 (reference)
Yes	99 (11.4)	16 (4.1)	33 (7.0)	49 (5.7)	2.5 (1.73-3.64)
Alcohol consumption					
<1/mo	746 (75.4)	417 (71.5)	341 (78.9)	758 (74.7)	1.0 (reference)
≤1-3/mo	161 (16.3)	131 (22.5)	49 (11.3)	180 (17.7)	0.8 (0.64-1.05)
≥1/wk	82 (8.3)	35 (6.0)	42 (9.7)	77 (7.6) [†]	1.0 (0.71-1.39)
Cigarette smoking					
<400 cigarettes/lifetime	894 (90.5)	536 (91.9)	445 (94.7)	981 (93.2)	1.0 (reference)
≥400 cigarettes/lifetime	94 (9.5)	47 (8.1)	25 (5.3)	72 (6.8)	1.5 (1.05-2.05)

NOTE: Abbreviation: LEE, lifetime estrogen exposure.

*OR of case versus all control adjusted for age, family history of breast cancer, and lifetime estrogen exposure.

[†]P < 0.05 by χ^2 test or Student's *t* test between hospital-based control and community control.[‡]Fx of BrCa, family history of breast cancer in first- and second-degree relatives.

For survival analysis, Kaplan-Meier analysis was used to assess the cumulative survival probabilities and differences were evaluated using the log-rank test. Hazard ratios (HR) were calculated with Cox proportional hazard model adjusted for age (years) and tumor-node-metastasis stage at diagnosis (stage I or II versus stage III or IV) for 529 breast cancer patients. All statistical analyses were done using STATA version 8.0 (Stata Corp. LP, College Station, TX).

Table 2. The distribution of *SULT1A1* and *SULT1E1* genotypes and the respective breast cancer risks

	Case n (%)	Control n (%)	OR (95% CI)
<i>SULT1A1</i>			
c.779G>A			
GG	796 (80.7)	830 (79.4)	1.0 (reference)
GA	190 (19.3)	215 (20.6)	0.9 (0.74-1.15)
AA	0 (0)	0 (0)	—
*14A>G			
AA	796 (80.7)	822 (78.8)	1.0 (reference)
AG	190 (19.3)	220 (21.1)	0.9 (0.71-1.10)
GG	1 (0.1)	1 (0.1)	—
*85C>T			
CC	599 (61.0)	606 (59.7)	1.0 (reference)
CT	365 (37.2)	388 (38.2)	1.0 (0.81-1.17)
TT	18 (1.8)	21 (2.1)	0.8 (0.42-1.55)
<i>SULT1E1</i>			
IVS1-447 C>A			
CC	559 (58.2)	581 (58.9)	1.0 (reference)
CA	348 (36.2)	344 (34.9)	1.0 (0.85-1.26)
AA	54 (5.6)	61 (6.2)	1.0 (0.64-1.42)
IVS4-1653 T>C			
TT	843 (85.4)	890 (85.4)	1.0 (reference)
TC	135 (13.7)	143 (13.7)	1.0 (0.77-1.28)
CC	9 (0.9)	9 (0.9)	1.0 (0.37-2.71)
*959G>A			
GG	536 (54.3)	511 (49.5)	1.0 (reference)
GA	383 (38.8)	434 (42.0)	0.9 (0.71-1.03)
AA	69 (7.0)	88 (8.5)	0.7 (0.53-1.05)*
GA/AA	452 (45.7)	522 (50.5)	0.8 (0.70-1.00)

NOTE: OR adjusted for age, family history of breast cancer, and lifetime estrogen exposure.

*P_{trend} = 0.035.

Results

As shown in Table 1, family history of breast cancer in first- and second-degree relatives (OR, 2.5; 95% CI, 1.60-4.02), lifetime estrogen exposure (per 5 years; OR, 1.3; 95% CI, 1.18-1.45), use of HRT (OR, 2.5; 95% CI, 1.73-3.64), and smoking (OR, 1.5; 95% CI, 1.05-2.05) increased the risk of breast cancer significantly after adjusting by age, family history of breast cancer in first- and second-degree relatives, and lifetime estrogen exposure. Although community controls were older than hospital controls, the distributions of most known risk factors (e.g., education, family history of breast cancer in first- and second-degree relatives, use of OC, and use of HRT) were not significantly different (Table 1).

Table 3. Haplotype frequencies of *SULT1A1* and *SULT1E1* in cases and controls

Haplotype			Case (%)	Control (%)	P (χ^2 test)	
<i>SULT1A1</i>						
c.779G>A						
	*14A>G	*85C>T				
G	A	C	79.6	78.8	0.494	
G	A	T	10.7	10.9		
G	G	C	<0.1	0.1		
G	G	T	0.1	0.2		
A	A	C	<0.1	0.2		
A	A	T	<0.1	<0.1		
A	G	T	9.6	9.8		
<i>SULT1E1</i>						
IVS1-447C>A						
C	IVS4-1653 T>C	*959G>A				
C	T	G	46.6	48.1	0.160	
C	T	A	22.1	20.7		
C	C	G	1.5	1.4		
C	C	A	6.0	6.1		
A	T	G	22.8	22.9		
A	T	A	0.9	0.8		
A	C	G	<0.1	<0.1		
A	C	A	<0.1	<0.1		

NOTE: Estimated by Bayesian methods using the Phase 2.0.2 program.

Table 4. The distribution of *SULT1A1* and *SULT1E1* diplotypes and the respective breast cancer risks

	Case	Control	OR (95% CI)
<i>SULT1A1</i> *			
GAC-GAC	597 (61.0)	598 (59.6)	1.0 (reference)
GAC-AGT	173 (17.7)	184 (18.3)	0.9 (0.74-1.21)
GAC-GAT	189 (19.3)	193 (19.2)	1.0 (0.80-1.28)
GAT-AGT	14 (1.4)	18 (1.8)	0.7 (0.33-1.39)
Others	6 (0.61)	11 (1.1)	0.6 (0.23-1.78)
Total	979 (100.0)	1,004 (100.0)	
<i>SULT1E1</i> †			
CTG-CTG	234 (24.4)	218 (22.2)	1.0 (reference)
CTG-CTA	176 (18.3)	217 (22.1)	0.8 (0.59-1.03)
CTG-CCA	73 (7.6)	52 (5.3)	1.4 (0.90-2.05)
CTG-ATG	224 (23.3)	195 (19.8)	1.1 (0.80-1.38)
CTA-CTA	43 (4.5)	37 (3.8)	1.1 (0.64-1.72)
CTA-CCA	15 (1.6)	32 (3.3)	0.5 (0.24-0.88)
CTA-ATG	91 (9.5)	111 (11.3)	0.8 (0.57-1.13)
CCA-CCA	5 (0.5)	6 (0.6)	0.9 (0.24-3.02)
CCA-ATG	24 (2.5)	23 (2.3)	0.9 (0.49-1.66)
ATG-ATG	50 (5.2)	58 (5.9)	0.8 (0.55-1.30)
Others	25 (2.6)	34 (3.5)	0.7 (0.39-1.22)
Total	960 (100.0)	983 (100.0)	

NOTE: Adjusted for age, family history of breast cancer, and lifetime estrogen exposure.

*Combination of *SULT1A1* haplotypes reconstructed from *SULT1A1* c.779G>A, *14A>G, and *85C>T.

†Combination of *SULT1E1* haplotypes reconstructed from *SULT1E1* IVS1-447C>A, IVS4-1653T>C, and *959G>A.

The allele distribution in two of the six studied polymorphic loci (i.e., *SULT1A1* *14A>G and *85C>T) did not conform to the HWE.

The *SULT1E1* *959 A allele-containing genotypes (AA/AG) posed a significantly decreased breast cancer risk compared with the GG genotype (OR, 0.8; 95% CI, 0.70-1.00). A significant trend was seen between the number of the *SULT1E1* *959 A alleles and breast cancer risk ($P_{\text{trend}} = 0.035$). In contrast, no significant association was observed between the *SULT1A1* genetic polymorphisms nor between the *SULT1E1* IVS1-447C>A and IVS4-1653T>C polymorphisms and breast cancer risk (Table 2).

A significant interaction was observed between *SULT1E1* *959 genotypes and use of OCs; ever users of OCs carrying the *SULT1E1* *959 GG genotype had a 1.6-fold risk of breast cancer (95% CI, 1.09-2.23) compared with never users of OCs carrying the *SULT1E1* *959 A allele-containing genotypes (AA/AG); this interaction was mainly attributable to premenopausal women (OR, 2.2; 95% CI, 1.29-3.73; $P_{\text{interaction}} = 0.030$).

When estimating the *SULT1A1* haplotypes reconstructed from individual SNPs of *SULT1A1* c.779G>A, *14A>G, and *85C>T and the *SULT1E1* haplotypes reconstructed from *SULT1E1* IVS1-447C>A, IVS4-1653T>C, and *959G>A, a total of seven haplotypes were estimated from the genotype data for the *SULT1A1* and eight haplotypes for the *SULT1E1* (Table 3). Only seven of these haplotypes (three for *SULT1A1* and four for *SULT1E1*) exhibited a frequency of >5%. Overall, the haplotype distributions were not significantly different between the cases and controls ($P = 0.494$ for *SULT1A1* and $P = 0.160$ for *SULT1E1*). However, compared with the CTG-CTG diplotype of *SULT1E1*, consisting solely of the major alleles of each three polymorphic loci of the *SULT1E1* gene, the CTA-CCA diplotype, consisting of the homozygous *959 AA genotype together with the IVS4-1653 T>C base, posed a decreased risk of breast cancer (OR, 0.5; 95% CI, 0.24-0.88) suggesting that the association between breast cancer risk and *SULT1E1* polymorphisms was stronger in the haplotype analysis than in individual SNP analysis (*959G>A polymorphism only; Table 4).

The clinicopathologic characteristics of 529 patients for survival analysis are shown in Table 5. No significant differences in the genotype frequency were seen between the tumor-

node-metastasis stage or ER/PR status (data not shown). However, the *SULT1E1* IVS4-1653 T allele-containing genotypes (TC/CC) were related to better survival (Table 6), particularly among women with ER-negative breast cancer (HR, 3.3; 95% CI, 1.20-8.99 with ER negative); the overall adjusted hazard ratio for disease-free survival associated with the TC/CC genotype was 3.2 (95% CI, 1.39-7.48). Moreover, the CTG-CCA diplotype increased hazard of disease-free survival into >4-fold compared with the referent CTG-CTG diplotype (HR, 4.2; 95% CI, 1.15-15.15; Table 6).

Discussion

When the six common SNPs were explored separately in the study, only the *SULT1E1* *959G>A polymorphism seemed to modulate the individual breast cancer risk in Korean women, and this association was only very moderate and of borderline significance. Consistent with our finding, Seth et al. (6) reported a similar lack of significant overall association between the *SULT1A1* genotypes and breast cancer risk in a study involving 444 cases and 227 controls. Zheng et al. (7), on the other hand, found that the *SULT1A1* c.779 A allele increased the risk of breast cancer in a nested case-control study with 156 cases and 332 controls, and Tang et al. (8) found a tendency of inverse association in a study on 103 cases and 230 controls. Recently, Han et al. (9) also reported a positive association between the *SULT1A1* c.779 A allele and breast cancer risk in a Chinese study involving 213 cases and 430 controls. Potential reasons for these discrepancies include differences in the allele frequencies, sample size, and ethnicity. The present study had >84% power (two-sided test of significance, ≤ 0.05) to detect an OR of 1.5 for carriers of *SULT1A1* c.779 A allele with the frequency of 0.1.

There seems a good biological explanation for the present observations. Falcony et al. (4) did not detect the *SULT1A1* protein in human mammary epithelial cell, which corresponds with the absence of *SULT1A1* activity in human mammary epithelial cytosol. *SULT1A1* also have an affinity for β -estradiol (E2) sulfation 700- to 3,000-fold lower than that of *SULT1E1* (22), suggesting that estrogen sulfation by *SULT1A1* in human

Table 5. Clinical characteristics of patients in survival analysis

Characteristics	No. event, n (%)	No. recurrence/death, n (%)
No. subjects	529	27
Median age at diagnosis, y (range)	45 (22-82)	44 (34-76)
Stage (AJCC)		
I	23 (4.3)	0 (0.0)
II	475 (89.0)	21 (80.8)
III	27 (5.1)	3 (11.5)
IV	9 (1.7)	2 (7.7)
T stage		
0	23 (4.3)	0 (0.0)
I	244 (45.6)	9 (34.6)
II	234 (43.7)	13 (50.0)
III	24 (4.5)	2 (7.7)
IV	10 (1.9)	2 (7.7)
Lymph node status		
Negative	331 (61.8)	6 (22.2)
Positive	205 (38.2)	21 (77.8)
Distant metastasis	9 (1.7)	2 (7.7)
ER		
Positive	316 (62.1)	7 (29.2)
Negative	193 (37.9)	17 (70.8)
PR		
Positive	230 (45.2)	4 (16.7)
Negative	279 (54.8)	20 (83.3)
Median F/U duration, mos (range)	13.6 (3.8-73.3)	17.4 (3.8-28.3)

Table 6. Disease-free survival of breast cancer patients by *SULT1E1* genotype and diplotypes

	No. patients	Person-time	Recurrence/death	Adjusted hazards ratio (95% CI)
Genotypes				
IVS1-447C>A				
CC	306	5,400.3	17	1.0 (reference)
CA + AA	220	4,263.3	10	0.7 (0.30-1.50)
IVS4-1653 T>C				
TT	457	8,485.2	19	1.0 (reference)
TC + CC	70	1,251.7	8	3.2 (1.39-7.48)
*959G>A				
GG	295	5,521.1	14	1.0 (reference)
GA+AA	233	4,281.3	13	1.3 (0.58-2.72)
Haplotypes*				
CTG-CTG	121	2,140.8	4	1.0 (reference)
CTG-CTA	107	2,052.8	5	1.2 (0.29-4.99)
CTG-CCA	39	703.6	6	4.2 (1.15-15.15)
CTG-ATG	137	2,702.3	8	0.9 (0.22-3.33)
CTA-CTA	22	229.2	1	7.9 (0.77-81.87)
CTA-CCA	8	132.8	0	—
CTA-ATG	41	826.1	0	—
CCA-CCA	3	45.7	0	—
CCA-ATG	8	148.0	1	2.1 (0.21-19.84)
ATG-ATG	26	474.5	1	1.6 (0.17-14.35)
Others	13	197.6	1	3.0 (0.77-6.93)
Total	529	9,831.7	27	

NOTE: Hazards ratio adjusted for age and tumor-node-metastasis stage (I or II versus III or IV).

*Combination of *SULT1E1* haplotypes reconstructed from *SULT1E1* IVS1-447C < A, IVS4-1653T > C, and *959G > A.

mammary epithelial cell line does not alter E2 levels at which E2 interacts with the ER.

We also analyzed the gene-environment interaction between genetic polymorphisms of *SULT1A1* or *SULT1E1* and estrogen exposure (i.e., use of OCs, HRT, and lifetime estrogen exposure). Although the OC use itself did not significantly increase the overall breast cancer risk (OR, 1.2; 95% CI, 0.95-1.59) nor the risk in premenopausal women (OR, 1.4; 95% CI, 0.95-2.01), and although the prevalence of OC use was only about 13% in controls (30-40% in other studies), the risk was of similar magnitude as what was seen in a meta analysis of 54 studies (relative risk \pm SD = 1.17 \pm 0.081; ref. 30). The trend of increased risk of breast cancer in the younger women by OC observed in this study is also supported by previous findings (31). Although we observed a significant multiplicative interaction between use of OCs and the possession of the *SULT1E1* *959 GG genotype in the premenopausal women, HRT, and lifetime estrogen duration, which were stronger risk factor of breast cancer development, were not modified by the *SULT1A1* and *SULT1E1* genotypes. Thus, these results need to be interpreted cautiously.

The *SULT1E1* IVS4-1653 TC/CC genotype seemed significantly associated with decreased survival rate from breast cancer. The *SULT1E1* expression has previously been found to be significantly associated with improved prognosis and with decreased cell proliferation in MCF-7 cells (23, 24). These findings may support our results if *SULT1E1* IVS4-1653 TC-CC genotype decreased the expression levels. However, because data on the genotype to phenotype relation of the variant alleles of *SULT1E1* is not available, the underlying biological mechanism for our results is most speculative.

We also observed the discrepancy of the role of specific SNPs of *SULT1E1* on the risk and survival of breast cancer (e.g., *959G>A for risk and IVS4-1653T>C for survival). Although the discrepancy in certain germ line mutations (e.g., ATM and p73) between cancer development and prognosis is reported (32, 33), further studies on the effect of the polymorphisms on *SULT1E1* mRNA production or stability are needed to clarify the mechanisms of action underlying the associations with the risk and survival of breast cancer.

In contrast to *SULT1E1* polymorphism, no relation between the *SULT1A1* genotypes and breast cancer survival was seen in this study. This disagrees with the findings of Nowell et al. (34)

indicating a relationship between *SULT1A1* c.779 A>G polymorphism and survival in 337 tamoxifen-treated breast cancer patients. The latter result, however, contradicts the hypothesis that the lower *SULT1A1* activity conferred by the *SULT1A1* c.779 A allele would result in decrease clearance of 4-OH tamoxifen and thereby increase the efficacy of tamoxifen treatment.

Estimation of haplotypes from the separate SNP data has recently been commonly incorporated in the studies on gene environment interactions in environmentally induced diseases. In spite that the relatively common SNPs were selected (>10%) to increase the power of the study, we could not take full advantage of the haplotype analysis due to insufficient sample size, especially in the survival analysis. However, the haplotype analysis revealed more profound associations between the *SULT1E1* gene and both breast cancer risk and disease-free survival. The validity of haplotype analysis was supported by the high repeatability of genotype data (>95%) and the minimal ambiguity for the most haplotype data (precision, \geq 0.9).

Our study has several limitations. First, the controls were recruited from different sources (i.e., hospital-based patients or healthy women in community). Although some selected characteristics (e.g., age, menopausal status, and frequency of alcohol consumption) were different between the hospital-based and community controls, the distribution of most known risk factors and genotype frequencies were not different. The result did not change when the analysis was conducted after excluding the subjects with benign breast disease from the control group (data not shown). Second, two of the *SULT1A1* SNPs genotyped in this study were not in HWE. However, comparability analyses of the genotyping methods, in which 50 of randomly selected samples were tested with sequencing directly, gave 100% identical results. Because the Bayesian method used for estimating haplotypes in this study is not under the assumption of HWE, the departures from HWE may not significantly affect the haplotype estimation of *SULT1A1* (29).

In summary, our results suggest that genetically determined *SULT1E1*-related estrogen sulfation capacity could contribute to the development of breast cancer and disease free survival in Korean women.

Appendix A *SULT1A1* and *SULT1E1* primer and probe sequences

SNP ID	Locus	Class	5'	Sequence	3'
<i>SULT1A1</i>					
c.779A>G	Exon 7, His213Arg	Taqman probe Taqman probe Forward primer Reverse primer	FAM VIC	AGGGAGTGCCCCAC AGGGAGCGCCCCA GAACGACGTGTGCTGAACCA GGAGATTCAAAAGATCCTGGAGTTT	MGBNFQ MGBNFQ
*14A>G	3' untranslated region	Taqman probe Taqman probe Forward primer Reverse primer	FAM VIC	TCCTGGAGTCACTGC CTCCTGGGGTCACT TTCCGCTCTGAGCTGTGAGA GGTCAGGTTTGATTGCGACACT	MGBNFQ MGBNFQ
*85C>T	3' untranslated region	Taqman probe Taqman probe Forward primer Reverse primer	FAM VIC	TTGAGGGCCCGGA ATTGAGGGCCTGGGA TGACCAAGCGGCTCAAGAA CACCTCAGCTCCAAATTGC	MGBNFQ MGBNFQ
<i>SULT1E1</i>					
IVS1-447C>A	Intron 1	Taqman probe Taqman probe Forward primer Reverse primer	FAM VIC	TAAGTGAAAAAAAATAG TAAGTGGCAAAAAA TTGTCAACATTTTTTAAACAAGTGGC TTTTTGCTAAACAGAGCTATATTTGAATTT	MGBNFQ MGBNFQ
IVS4-1653T>C	Intron 4	Taqman probe Taqman probe Forward primer Reverse primer	FAM VIC	AGTAGATAACATAAAATAAC TAGTAGATAATATAAACTAG AAGTGATAAAGAGAAGAAAGTGACAAAAAG ACAAACCAATGGCTCAAATCC	MGBNFQ MGBNFQ
*959A>G	3' untranslated region	Taqman probe Taqman probe Forward primer Reverse primer	FAM VIC	TCTGCCTTGGTATTT CTGCCTTAGTATTTG ACATCCAAATGCAGAAGTGGTT TGTAAGAAAATGATCTAGTTTATATCTC	MGBNFQ MGBNFQ

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