



Report

Reproductive factors, glutathione S-transferase M1 and T1 genetic polymorphism and breast cancer risk

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Summary

We conducted a hospital-based case–control study to evaluate the interactive effect of reproductive factors and glutathione S-transferase (GST) M1 and T1 genetic polymorphisms in individual susceptibility to breast cancer. The study population consisted of 189 incident breast cancer cases and 189 age-matched controls with no known malignant diseases. *GSTM1/T1* genotypes were determined by a multiplex polymerase chain reaction (PCR) method, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by conditional logistic regression model. The parity factors were grouped as (1) high-risk status defined as nullipara or para with experience of first full-term pregnancy (FFTP) at or over 30 years, and (2) low-risk status defined as para with experience of FFTP under 30 years. A significant multiplicative interaction was observed between *GSTM1* and *GSTT1* null genotypes and high-risk status of parity factor in all women and in premenopausal women ($P \leq 0.01$), but not in postmenopausal women ($P > 0.05$). The interaction between the combined genotypes of *GSTM1* and *GSTT1* and status of parity factor was also significant in all women and in premenopausal women ($P < 0.01$). Our findings suggest that genetic polymorphisms *GSTM1/T1* could modify estrogen-related breast cancer risk.

Introduction

Breast cancer is the third most frequent cancer in Korean women and the incidence is increasing in both western countries and Korea [1, 2]. Although a substantial proportion of breast cancer cases are explained by well-established risk factors (i.e., later age at first birth, nulliparity, and first-degree family history of breast cancer) [3], the reason for the observed worldwide increase in breast cancer incidence is still largely unknown.

Epidemiological studies have suggested that environmental carcinogens may contribute to the increasing incidence of breast cancer [4]. Genetic differences

in the metabolism of environmental carcinogens may also be associated with individual variations in susceptibility to breast cancer [5, 6]. Glutathione S-transferases (GSTs) play an important role in the metabolism of environmental carcinogens like polycyclic aromatic hydrocarbons and heterocyclic aromatic amines, which are well-known mammary carcinogens [7]. GSTs also play an important role in the metabolism of estrogen and lipid peroxidation [8, 9].

Our previous study suggested that the *GSTM1* and *GSTT1* genotypes are important modifiers of susceptibility to breast cancer among premenopausal women but of less importance among postmenopausal women

Table 1. Selected characteristics for breast cancer cases and age-matched controls

Factors	All women		Premenopausal women		Postmenopausal women	
	Cases (n = 189)	Controls ^a (n = 189)	Cases (n = 114)	Controls (n = 104)	Cases (n = 75)	Controls (n = 81)
Age	47.8 (11.3)	48.3 (11.4)	40.8 (6.0)	40.5 (6.3)	58.0 (9.0)	58.4 (9.0)
Education (over college) (%)	28.2 ^b	14.4	35.4 ^b	17.6	19.3 ^b	10.1
Age at menarche (SD)	15.3 (1.6)	15.5 (1.8)	15.1 (1.5)	14.9 (1.7)	16.0 (1.7)	15.9 (1.7)
Age at menopause (SD)	48.9 (4.7)	47.7 (6.2)	–	–	48.9 (4.7)	47.7 (6.2)
FTP ^c (%)	91.0	96.3	92.2	97.1	89.3 ^b	95.1
Age at FFTP ^d in parous women (SD)	26.3 (3.9) ^b	24.5 (3.4)	26.9 (3.9) ^b	25.2 (3.1)	25.3 (3.6) ^b	23.6 (3.5)
Regular menstruation (%)	87.3	83.5	83.3	79.8	93.3	87.5
Use of oral contraceptives (%)	7.4	8.5	5.2	9.6	10.7	7.4
BMI ^e (SD)	23.3 (3.3)	23.2 (3.2)	22.6 (3.0)	22.8 (3.1)	24.2 (3.6)	23.8 (3.3)
Smoking (%)	5.3	7.9	4.4	5.6	6.6 ^b	10.1
Drinking (%)	27.5 ^b	16.9	33.3	25.0	18.7 ^b	7.4
Family history (%)	10.1 ^b	4.2	7.9	4.8	13.3 ^b	3.7

^a Four controls were managed to the missing in menopausal status.

^b $P < 0.05$.

^c FTP, full-term pregnancy.

^d FFTP, first full-term pregnancy.

^e BMI, body mass index.

[10]. Here we extended the study to evaluate a hypothesis that the women who are lacking both of the *GST* genes, involved in inactivation of reactive endogenous and exogenous estrogens, may be at the higher risk of developing breast cancer when they have high-risk status of parity factors such as nullipara or later age at first full-term pregnancy (FFTP).

Materials and methods

Study subjects

The study population consisted of a consecutive series of breast cancer patients and non-cancer controls admitted between March 1994 and September 1998 to three teaching hospitals located in Seoul, Korea. The study design and selection of study subjects has been described in detail elsewhere [10]. Incident breast cancer cases who are confirmed by histopathological diagnosis and from whom a blood sample was available were selected as cases ($n = 204$). Controls ($n = 332$), who were individuals with no present or previous history of breast cancer, were simultaneously recruited in the same hospitals. Women with amenorrhea, previous history of hysterectomy, oophorectomy, hormone replacement therapy, and hormone-related diseases such as thyroid problems were excluded from both groups. Benign breast tumor, other breast diseases

(e.g., mastitis and benign calcification), and other systemic problems like chronic liver diseases were also excluded from the controls. According to the above criteria, 189 cases and 233 controls were chosen to be eligible for the study. Each patient was then frequency-matched to one control according to the following age groups: under 29, 30–34, 35–39, 40–54, 55–69, and over 70 years. Consequently, the final study population consisted of 189 cases and 189 controls.

Informed consents were obtained at the time of blood collection. Information on demographic characteristics, education, marital status, family history of breast cancer in the first and second relatives, reproductive factors and menstruation, life style habits including alcohol consumption, duration of alcohol drinking, and diet were collected using a questionnaire administered by trained interviewers.

Genotyping

DNA was isolated using standard methods from blood drawn into 10 ml heparinized tubes and stored in -20°C until use. The *GSTM1* and *GSTT1* genotypes were determined by a multiplex polymerase chain reaction (PCR) method as described in Refs. [11–13]. Briefly, successful amplification by β -globin specific primers confirmed the proper function of the PCR reaction, whereas presence or absence of the *GSTM1*

Table 2. Status of parity factor for breast cancer cases and age-matched controls

Factors	All women			Premenopausal women			Postmenopausal women		
	Cases (n = 189)	Controls (n = 189)	OR ^a (95% CI)	Cases (n = 114)	Controls (n = 104)	OR ^a (95% CI)	Cases (n = 75)	Controls (n = 81)	OR ^b (95% CI)
Parity factor									
Nulliparous	6 (3.4)	4 (2.1)	2.9 (0.7–12.0)	3 (2.8)	0 (0.0)	–	3 (4.3)	4 (2.1)	1.2 (0.2–6.7)
Age at FFTP ^b									
≤19	4 (2.2)	10 (5.4)	0.7 (0.2–2.6)	2 (1.9)	2 (2.0)	2.7 (0.2–31.8)	2 (2.9)	7 (5.4)	0.4 (0.1–2.3)
20–24	57 (32.0)	82 (44.1)	1.0 (reference)	27 (25.0)	38 (37.6)	1.0 (reference)	30 (42.9)	43 (44.1)	1.0 (reference)
25–29	84 (47.2)	79 (42.5)	1.4 (0.8–2.3)	55 (50.9)	54 (53.5)	1.5 (0.7–2.9)	29 (41.4)	24 (42.5)	1.5 (0.7–3.3)
30–34	21 (11.8)	9 (4.8)	2.5 (1.0–6.1)	16 (14.8)	7 (6.9)	2.3 (0.8–6.5)	5 (7.1)	1 (4.8)	7.2 (0.8–66.3)
≥35	6 (3.4)	2 (1.1)	3.7 (0.7–19.7)	5 (4.6)	0 (0.0)	–	1 (1.4)	2 (1.1)	0.6 (0.0–7.3)
Missing	11	3	–	6	3	–	5	–	–
Test for trend in parous women			<i>P</i> < 0.01			<i>P</i> < 0.01			<i>P</i> = 0.10
Two risk groups according to parity factor ^c									
Low-risk	145 (81.5)	171 (91.9)	1.0 (reference)	84 (77.8)	94 (93.1)	1.0 (reference)	61 (87.1)	74 (91.4)	1.0 (reference)
High-risk	33 (18.5)	15 (8.1)	2.4 (1.2–4.6)	24 (22.2)	7 (6.9)	2.8 (1.1–6.9)	9 (12.9)	7 (8.6)	1.9 (0.6–5.7)

^a OR was adjusted for age (categorical variable) and education.

^b FFTP, first full-term pregnancy.

^c Low-risk status was defined as the experience of FFTP under 30 years and high-risk status was defined as the nullipara or the experience of FFTP at or over 30 years.

Table 3. The association between combined genotypes of *GSTM1* and *GSTT1* and breast cancer

	All women			Premenopausal women			Postmenopausal women		
	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR ^a (95% CI)	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR ^a (95% CI)	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR ^a (95% CI)
<i>GSTM1/T1</i>									
No null	32 (17.0)	48 (26.5)	1.0 (reference)	17 (14.9)	23 (23.7)	1.0 (reference)	15 (20.3)	28 (28.8)	1.0 (reference)
One null	108 (57.5)	95 (52.5)	1.7 (1.0–3.1)	66 (57.9)	58 (59.8)	2.1 (0.9–4.5)	42 (56.7)	36 (45.0)	1.8 (0.7–4.4)
Two null	48 (25.5)	38 (21.0)	2.2 (1.1–4.4)	31 (27.2)	16 (16.5)	4.4 (1.6–11.9)	17 (23.0)	21 (26.3)	1.2 (0.4–3.6)
<i>P</i> for trend ^b			<i>P</i> = 0.03			<i>P</i> = 0.01			<i>P</i> > 0.10

^a The ORs were adjusted for age, education, body mass index, age at menarche, age at first pregnancy, age at menopause, duration of breast-feeding, family history of breast cancer, and menopausal status at baseline.

^b *P* for trend for both positive, either null, and both nulls.

Table 4. The interaction of *GSTM1* genotype and status of parity factor for breast cancer

	Status of parity factor		<i>P</i> for interaction
	Low-risk ^a	High-risk ^b	
All women			
<i>GSTM1</i> -positive	1.0 (reference)	2.3 (0.9–5.6)	<i>P</i> = 0.01
<i>GSTM1</i> -null	1.2 (0.8–1.8)	3.1 (1.2–7.8)	
Premenopausal women			
<i>GSTM1</i> -positive	1.0 (reference)	3.1 (0.9–10.6)	<i>P</i> < 0.01
<i>GSTM1</i> -null	1.6 (0.9–2.8)	6.8 (1.8–25.2)	
Postmenopausal women			
<i>GSTM1</i> -positive	1.0 (reference)	1.9 (0.4–8.4)	<i>P</i> = 0.88
<i>GSTM1</i> -null	0.8 (0.4–1.5)	0.7 (0.1–3.5)	

^a Low-risk status was defined as the experience of FFTP under 30 years.

^b High-risk status was defined as the nullipara or the experience of FFTP at or over 30 years.

Table 5. The interaction of *GSTT1* genotype and status of full-term pregnancy for breast cancer

	Status of parity factor		<i>P</i> for interaction
	Low-risk ^a	High-risk ^b	
All women			
<i>GSTM1</i> -positive	1.0 (reference)	1.5 (0.7–3.3)	<i>P</i> < 0.01
<i>GSTM1</i> -null	1.2 (0.8–1.9)	10.3 (2.3–45.9)	
Premenopausal women			
<i>GSTM1</i> -positive	1.0 (reference)	2.9 (1.0–8.3)	<i>P</i> < 0.01
<i>GSTM1</i> -null	1.4 (0.8–2.5)	12.6 (1.6–102.1)	
Postmenopausal women			
<i>GSTM1</i> -positive	1.0 (reference)	0.4 (0.1–2.0)	<i>P</i> = 0.06
<i>GSTM1</i> -null	1.0 (0.5–2.0)	7.9 (0.9–67.3)	

^a Low-risk status was defined as the experience of FFTP under 30 years.

^b High-risk status was defined as the nullipara or the experience of FFTP at or over 30 years.

and *GSTT1* specific amplification products indicated the respective positive and null genotypes of the genes. The reliability of the PCR analyses conducted in the Korean laboratory was controlled by re-assaying 40 randomly selected samples in the Finnish laboratory; the results were found to be identical in both laboratories.

Statistical analyses

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by conditional logistic regression model. The parity factor was grouped as follows: (1) high-risk status defined as nullipara or para with experience of FFTP at or over 30 years, and (2) low-risk status defined as para with experience of FFTP under

30 years. Linear increase in the risk with exposure or genotype was evaluated by likelihood ratio test. And, the interaction between genetic polymorphism and the status of parity factor for breast cancer risk were tested by likelihood ratio test for adding the interaction term to the main effect model, containing genotype and the status of parity factor [14, 15].

Results

As shown in Table 1, age at interview, education, marital status, family history of breast cancer, age at menarche, age at menopause, the experience of FTP, age at FFTP, the experience of breast-feeding, and

Table 6. The interaction of the combination of *GSTM1* and *GSTT1* genotypes and status of parity factor for breast cancer

	Status of parity factor		<i>P</i> for interaction
	Low-risk ^a	High-risk ^b	
All women			
Both positive	1.0 (reference)	1.7 (0.6–5.2)	<i>P</i> < 0.01
Either null	1.5 (0.9–2.6)	3.2 (1.2–8.7)	
Both null	1.4 (0.7–2.7)	15.0 (1.8–122.6)	
<i>P</i> for trend	<i>P</i> = 0.15	<i>P</i> = 0.05	
Premenopausal women			
Both positive	1.0 (reference)	3.4 (0.7–15.4)	<i>P</i> < 0.01
Either null	1.6 (0.8–3.3)	4.7 (1.3–17.1)	
Both null	2.5 (1.0–6.1)	– ^c	
<i>P</i> for trend	<i>P</i> = 0.07	<i>P</i> = 0.14	
Postmenopausal women			
Both positive	1.0 (reference)	0.8 (0.1–5.5)	<i>P</i> = 0.28
Either null	1.4 (0.6–3.1)	1.6 (0.3–8.4)	
Both null	0.7 (0.3–1.9)	3.7 (0.3–38.9)	
<i>P</i> for trend	<i>P</i> = 0.89	<i>P</i> = 0.30	

^a Low-risk status was defined as the experience of FFTP under 30 years.

^b High-risk status was defined as the nullipara or the experience of FFTP at or over 30 years.

^c Not estimated because of zero cell.

duration of breast-feeding appeared as significant risk factors for breast cancer in Korean women, agreeing with previous observations in Caucasians [3].

Nulliparous women (OR = 2.9, 95% CI = 0.7–12.0), women who experienced FFTP at age of 30–34 years (OR = 2.5, 95% CI = 1.1–6.1), and women who experienced FFTP at age of over 34 years (OR = 3.7, 95% CI = 0.7–19.7), were at the significantly increased risk of breast cancer compared with those who experienced FFTP at age of 20–24 years. When the parity factors were grouped into high-risk and low-risk status, the risk of breast cancer in women with the high-risk status of parity factor was OR = 2.4 (95% CI = 1.2–4.6) for all women, OR = 2.8 (95% CI = 1.1–6.9) for premenopausal women, and 1.9 (95% CI = 0.6–5.7) for postmenopausal women (Table 2).

In our previous study [10] where the *GST* genes were studied separately, the *GSTM1* and *GSTT1* genotypes were found to be important modifiers of susceptibility to breast cancer among premenopausal women but of much less importance among postmenopausal women. In the present study, similar phenomena was found when the potential combined effect of the *GSTM1* and *GSTT1* genotypes was examined; concurrent lack of both the genes posed more than 4-fold

risk of premenopausal breast cancer (OR = 4.4, 95% CI = 1.6–11.9), whereas no significant effect was observed in the risk of postmenopausal breast cancer (OR = 1.2, 95% CI = 0.4–3.6) (Table 3).

When stratified by the parity factor, a significant interaction was observed between the *GSTM1* null genotype and high-risk status of parity factor among all women (*P* = 0.01), and premenopausal women (*P* < 0.01), but not among postmenopausal women (*P* = 0.88) (Table 4). A significant interaction was also observed between the *GSTT1* null genotype and high-risk status of parity factor among all women (*P* < 0.01) and premenopausal women (*P* < 0.01), and a marginally significant interaction among postmenopausal women (*P* = 0.06) (Table 5). Similarly, the interaction between the combined *GSTM1* and *GSTT1* genotype and status of parity factor was also significant in all women (*P* < 0.01) and in premenopausal women (*P* < 0.01), but not in postmenopausal women (*P* = 0.28) (Table 6).

The *GSTM1* null genotype accompanied with high-risk status of parity factor resulted in a 3.1-fold risk (95% CI = 1.2–7.8) of breast cancer, which was mainly attributable to premenopausal breast cancer (OR = 6.8, 95% CI = 1.8–25.2) (Table 4). The combined effect of *GSTT1* null genotype and high-risk

parity factor was much more striking; women with this combination of the risk factors were at 10.3-fold (95% CI=2.3–45.9) (Table 5). Again, this association was mainly attributable to premenopausal breast cancer (OR = 12.6, 95% CI = 1.6–102.1). The most remarkable risk was observed for women lacking both *GSTM1* and *GSTT1* genes and having the high-risk status of parity factor; they were at 15-fold risk for breast cancer compared with women with both the genes and the low-risk status of parity factor (OR = 15.0, 95% CI = 1.8–122.6) (Table 6).

Discussion

In this study, a significant interactive effect between the *GST* genotypes and high-risk parity factors was observed; women with high-risk status of parity factor, and concurrently lacking both of the genes were particularly vulnerable for developing breast cancer. Since GSTs can metabolize steroids, and lipid-peroxidation products, and free radicals leading to DNA damage [16, 17], which can indirectly be produced by estrogen metabolism [9, 18], it is conceivable that their genetic polymorphisms could modify estrogen-related breast cancer risk.

Estrogen can also cause genetic alteration and effect tumor initiation by direct binding on breast tissue [19]. Estrogen and estrogen metabolites (catechol estrogens) can also bind to DNA and trigger DNA damage [8, 18, 20–22]. Consequently, the nulliparous women and women who experienced FFTP at older age might both be exposed to higher active estrogen and consequently be more susceptible to endo/exogenous estrogen exposure on breast tissue and increased mitotic activity in the breast epithelium [23]. Although a few previous studies indicated the potential association between *GSTM1* and *GSTT1* genotypes and breast cancer risk [10, 24–26], none of them addressed their interactive effect with reproductive factors.

In many epidemiological studies, the experience of FTP has been asserted that it protects against breast cancer [27, 28]. However, women who had FFTP at later age were at the increased risk of breast cancer compared with nulliparous women. This cross-over effect was also seen in this study. The risk of nulliparous women (OR = 2.9, 95% CI = 0.7–12.0) was slightly higher than that of women with FFTP at age of 30–34 years (OR = 2.5, 95% CI = 1.0–6.1), but lower than in women with FFTP at age of over 34 years (OR = 3.7, 95% CI = 0.7–19.7). When the parity factors were

grouped into high-risk and low-risk status groups, the risk of breast cancer was 2.4-fold among women with the high-risk status of parity factor compared to the women with the low-risk parity factor. These observations agree with those previously found in another Asian study population [29]. Although the elevated risk related with later age of FFTP might result from increased estrogen exposure in breast tissue [30], the biological mechanism underlying these phenomena is still somewhat unclear.

Our findings therefore remain to be corroborated in future studies. The other susceptibility genes involved in estrogen metabolism like *COMT*, *CYP1B1*, and *CYP17*, need also to be explored in these studies to better understand the interactive effect between genetic and reproductive factors in breast cancer.

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